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(54) Title: A GENE RELATED TO MIGRAINE IN MAN

(57) Abstract

Genes for familial hemeplegic migraine (FHM), episodic ataxia type-2 (EA-2), common forms of migraine, and other episodic neurological disorders, such as epilepsy, have been mapped to chromosome 19p13 and chromosome 10p12. A brain-specific P/Q-type calcium channel subunit gene, covering 300 kb with 47 exons is provided. The exons and their surroundings reveal polymorphic variations and deleterious mutations that are linked to various types of calcium channel dysfunctions causing episodic neurological disorders in man or animals.

Title: A gene related to migraine in man.

Migraine is a frequent paroxysmal neuro-vascular disorder, characterized by recurrent attacks of disabling headache, vomiting, photo/phonophobia, malaise, and other general symptoms (migraine without aura). Up to 20% of patients may, in addition, experience transient neurological (aura) symptoms during attacks (migraine with aura) (HCC, 1988). Up to 24% of females and 12% of males in the general population are affected, however with variable attack frequency, duration and severity (Russell *et al.*, 1995). Knowledge about the mechanisms of the final common pathway of migraine attacks has increased substantially the last five years, resulting in improved, though still only symptomatic (and sub-optimal) acute treatment for the attack. There is, however, still very little knowledge about the *etiology* of migraine attacks, i.e. why and how attacks begin and recur. Accordingly, prophylactic treatment for migraine is non-specific and has only limited efficacy.

Family, twin and population-based studies suggest that genetic factors are involved in migraine, most likely as part of a multifactorial mechanism (reviewed by Haan *et al.*, 1996). The complex genetics has hampered identification of candidate genes for migraine. Familial Hemiplegic Migraine (FHM) is a rare, autosomal dominant, subtype of migraine with aura, associated with ictal hemiparesis and, in some families cerebellar atrophy (HCC, 1988). Otherwise, the symptoms of the headache and aura phase of FHM and "normal" migraine attacks are very similar and both types of attacks may alternate within subject and co-occur within families. FHM is thus part of the migraine spectrum and can be used as a model to study the complex genetics of the more common forms of migraine (Haan *et al.*, 1996). A gene for FHM has been assigned to chromosome 19p13 in about half of the families tested (Joutel *et al.*, 1993; Ophoff *et al.*, 1994;

Joutel *et al.*, 1995). Remarkably, cerebellar atrophy was found only in families with FHM linked to chromosome 19p13, but not in unlinked families. Recently, we showed the 19p13 FHM locus to be also involved in "normal" migraine (May *et al.*, 1995).

Episodic ataxia type 2 (EA-2) is another, autosomal dominant, paroxysmal neurological disorder, characterized by acetazolamide-responsive attacks of cerebellar ataxia and migraine-like symptoms, and interictal nystagmus and cerebellar atrophy. Recently, a gene for EA-2 was assigned to chromosome 19p13, within the same interval as for FHM (Kramer *et al.*, 1995). This finding, as well as the clinical similarities, raise the possibility of EA-2 and FHM being allelic disorders.

Since other hereditary episodic neurological disorders responding to acetazolamide (such as hypokalaemic and hyperkalaemic periodic paralysis), as well as EA type-1 (which, in contrast to EA-2, is associated with continuous myokymia and non-responsive to acetazolamide) have all been associated with mutations in genes encoding for ion channels (Ptacek *et al.*, 1991; Ptacek *et al.*, 1994; Brown *et al.*, 1994), we specifically looked for similar genes within the FHM and EA-2 candidate region.

In view of the above, the FHM/EA-2 locus can, since FHM is part of the migraine spectrum, thus be used to study the genetic factors and biological mechanisms that are related to various episodic neurological disorders such as FHM, EA-2, common migraine and others such as epilepsy.

Calcium channels are multisubunit complexes composed of at least an $\alpha1$, an $\alpha2\delta$, and a β subunit. The central $\alpha1$ subunit is functionally the most important component, acting as a voltage sensor and forming the ion-conducting pore. The other subunits have auxiliary regulatory roles. The membrane topology of the $\alpha1$ subunit consist of four hydrophobic motifs (I to IV), each containing six transmembrane α-helices (S1-

S6) and one hairpin (P) between S5-S6 that spans only the outer part of the transmembrane region.

The present invention provides an isolated and/or recombinant nucleic acid, or fragments thereof, encoding a Ca^{2+}-channel α1 subunit related to familial hemiplegic migraine and/or episodic ataxia type-2, derived from a gene present on chromosome 19p13.1-19p13.2; a gene encoding the α1 (ion-conducting) subunit of a P/Q-type voltage gated calcium channel. The present invention also provides access to and methods to study the genetic background and identify other subunits of the calcium channel subunit complexes and the proteins related therewith that are associated with the genetic factors and biological mechanisms that are related to various episodic neurological disorders such as FHM, EA-2, common migraine and others such as epilepsy which are related to cation channel dysfunction.

The sequence of the cDNA of the gene is highly related (≥90%) to a brain-specific rabbit and rat voltage gated P/Q-type calcium channel α1 subunit (Mori *et al.*, 1991; Starr *et al.*, 1991), and the open reading frame consists of 2261 amino acid residues. Northern blot analysis showed a brain-specific expression, especially in the cerebellum. Primary study of a cosmid contig harbouring the gene already indicated an exon distribution over at least 300 kb of genomic DNA. Recently, a neuronal Ca^{2+} α1A subunit gene was localized to chromosome 19p13.1-p13.2 by FISH analysis (Diriong *et al*, 1995). The gene symbol is CACNL1A4 and the α1 subunit is classified as a P/Q-type. No sequence data for the CACNL1A4 gene have been provided by Diriong or others, but the same localization (chromosome 19p13.1) and the identical classification (P/Q-type) suggests that the Ca^{2+} channel α1 subunit we have identified is very similar to CACNL1A4. No relation with migraine has been disclosed for CACNL1A4. The genomic structures of three other human Ca^{2+} channel α1 subunit genes (CACNL1A1, CACNL1A2 and CACNL1A3) have been published to date (Hogan et al, 1994; Soldatov, 1994; Yamada et al, 1995). Both CACNL1A1 and CACNL1A2 span

about 150 kb and consist of 50 and 49 exons, respectively. The smaller CACNL1A3 gene is composed of 44 exons, distributed over 90 kb.

The present invention also provides an isolated and/or recombinant nucleic acid comprising alleles of the invented gene which contain mutations relevant to the occurence of migraine and other neurological disorders which are related to cation channel dysfunction. Such mutations are for example a mutation at codon 192 resulting in the replacement of arginine by glutamine (R192Q), and/or a mutation at codon 666 resulting in the replacement of threonine by methionine, and/or a mutation at codon 714 resulting in a replacement of valine by alanine and/or a mutation at codon 1811 resulting in a replacement of isoleucine by leucine, but also other mutations of alleles of said gene which bear relationships with cation channnel dysfunction.

The present invention also provides isolated and/or recombinant nucleic acid comprising alleles of said gene which contain a polymorphic CA-repeat sequence specific for various alleles of said gene. The present invention also provides isolated and/or recombinant nucleic acids comprising alleles of said gene which contain a CAG repeat.

The present invention also provides methods and tests (such as PCR, but also other tests to detect or amplify nucleic acids are known in the art) to detect, identify and localize or distinguish genes and alleles of such genes, or fragments thereof, encoding for proteins or α, β or χ sub-units of specific *cerebral cation channels,* more specifically the invented gene and its various alleles encoding the $\alpha 1$ subunit of a P/Q-type voltage gated calcium channel and the gene encoding the $\beta 2$ sub-unit, which are involved in the primary pathogenesis of neurological disorders such as FHM, migraine, EA-2 and SCA6. With such methods and tests one can study abnormalities of said gene.

The invention also provides recombinant expression vectors comprising isolated and/or recombinant nucleic acid comprising alleles of said genes or fragments therof, and

provides host cells or animals that comprise such vectors or that are otherwise transformed with an isolated and/or recombinant nucleic acid according to the invention.

The invention thus also provides a rationale and methods for the testing and the development of specific prophylactic medication for migraine and other episodic neurological, in particular brain, disorders, such as epilepsy, associated with cation channel dysfunction.

The invention for example provides cells or animals that comprise recombinant vectors that comprise variants of said genes or cells or animals that are transformed with said variants. Also, the invention provides means to identify naturally occuring variants of experimental animals with changes in said gene related to FHM, EA-2, SCA7, migraine or other neurological disorders associated with cation channel dysfunction. An example of such an animal is the tottering mouse, and its variants called leaner and rolling, described in the experimental part of the invention. The invention also provides cells or animals in which changes such as deletions or mutations in said gene have been introduced by recombinant nucleic acid techniques. All such cells or animals provided by the invention can be used to study the pathophysiology of FHM, EA-2, migraine or other neurological disorders associated with cation channel dysfunction, for example to test or develop specific medication for the treatment of said disorders.

The invention also provides proteins or peptides encoded by said genes, or fragments thereof, related with cation channel dysfunction, and detection of such proteins or peptides by antibodies directed against said proteins or peptides. Such antibodies can be of natural or synthetic origin, and can be produced by methods known in the art. Such proteins and antibodies and detection methods can be used to further *in vitro* or *in vivo* studies towards the pathophysiology of FHM, EA-2, migraine or other neurological disorders associated with cation channel dysfunction, in addition such proteins, antibodies and detection methods can

also be used to diagnose or identify such disorders in patients or in experimental animals.

Experimental Procedures

Subjects

Sixteen FHM patients were selected, including eight individuals from four unrelated chromosome 19-linked FHM families (*NL-A, UK-B, USA-C* (Ophoff *et al*, 1994), and *USA-P* (Elliot *et al*., 1995), two affected individuals from two small FHM families from Italy (*Italy I & II*) and six individuals with sporadic hemiplegic migraine (i.e. no other family member was shown to suffer from attacks of hemiplegic migraine). In families *NL-A* and *USA-P* cerebellar ataxia and/or nystagmus is associated with FHM. An additional set of four subjects from four unrelated EA-2 families linked to chromosome 19, was also included (CAN-25, -45, -191, -197. Fifty randomly collected individuals from the Dutch population (Smith *et al*., 1988) were used as a control to determine the allele frequencies of polymorphic sites.

Patients with migraine with or without aura were diagnosed according to the international Headache Society (IHS) classification criteria. Patients attending the neurology outpatient clinic of Leiden University Medical Center, The Netherlands, and patients responding to calls in local newspapers or in the periodical of the Dutch Migraine Patients Association, were screened for a positive family history of migraine. Only families with migraine in at least two generations were asked to participate. Probands (n=36) and relatives (n=492) were personally examined and interviewed using semi-structured questionnaires. The questionnaire included questions about age at onset, frequency and duration of attacks, aura symtoms, premonitory signs and symptoms, triggers for attacks, medication, and additional headaches. When family members were not available for a personal interview, information on their migraine was collected by interviewing their relatives. Because of the low

validity of diagnosing migraine auras through relatives, we only assessed the presence or absence of migraine headaches. Whenever possible, medical records were examined.

Genomic structure

Ten different cosmids from the contig extending the invented gene, were subcloned separately in either M13 or pBlueScript KS vector. From each cosmid library at least 3x96 random clones with an average insert size of about 2 kb, were picked. Positive clones were identified by hybridization techniques and subsequently sequenced with vector-specific primers; intron-exon boundary sequences were completed using cDNA-based primers.

Mutation analysis, DHPLC and SSCP

Genomic DNA was used as template to generate polymerase chain reaction (PCR) products for single-strand conformational polymorphism (SSCP) analysis and denaturing high-performance liquid chromatography (DHPLC). Amplifications were performed in standard conditions with primer pairs as listed in Table 1 or listed below. Except for the 5' side of exon 6, primers were chosen to produce fragments that contained a single exon and at least 35 basepairs (including primer) of each flanking intron sequence. Amplification of exons 1 and 20 was performed producing two overlapping fragments and exon 19 was amplified into three overlapping fragments. In addition, the following markers;

D10S191	Primer sequence 1	CTT TAA TTG CCC TGT CTT C
	Primer sequence 2	TTA ATT CGA CCA CTT CCC
D10S245	Primer sequence 1	AGT GAG ACT CGT CTC TAA TG
	Primer sequence 2	ACC TAC CTG AAT TCC TGA CC
D10S89	Primer sequence 1	AAC ACT AGT GAC ATT ATT TTC A
	Primer sequence 2	AGC TAG GCC TGA AGG CTT CT

DHPLC (Oefner *et al.*, 1995; Hayward *et al.*, 1996) was carried out on automated HPLC instrumentation. Crude PCR products, which had been subjected to an additional 3-minute 95°C denaturing step followed by gradual reannealing from 95-65°C over a period of 30 minutes prior to analysis, were eluted with a linear acetonitrile (9017-03, J.T. Baker, Phillipsburg, N.J., USA) gradient of 1.8% per minute at a flow-rate of 0.9 ml/min. The start- and end-points of the gradient were adjusted according to the size of the PCR products (Huber *et al.*, 1995). The temperature required for successful resolution of heteroduplex molecules was determined empirically by injecting one PCR product of each exon at increasing mobile phase temperatures until a significant decrease in retention was observed.

For SSCP analysis, primary PCR products were labeled by incorporation of [α-^{32}P]dCTP in a second round of PCR. Samples were diluted and denatured in formamide buffer before electrophoresis. SSCP was carried out according to published protocols (Orita *et al.*, 1989; Glavac *et al.*, 1994). Digestion of several exons to yield products suitable for SSCP analysis.

Sequencing of PCR products was performed with an ABI 377 automated sequencing apparatus with cycle sequencing according to the manufacturer. Furthermore, PCR products were cloned in the TA vector (Invitrogen) and subjected to manual dideoxy sequence analysis (T7 Sequencing kit, Pharmacia Biotech.).

A total of 481 blood samples were collected from patients with migraine. Genomic DNA was isolated as described by Miller et al., 1988. The analyses of the highly informative microsatellite markers D19S391, D19S394, D19S221 and D19S226, D10S191, D10S245 and D10S89 were performed by PCR; primer sequences related to these markers are available through the human Genome Data Base (GDB).

The relative frequencies of marker alleles were estimated on the entire family material, with the relevant

correction for genetic relationships between individuals (Boehnke, M, 1991) with the ILINK option of the I-INKAGE package, version 5.03 (Lathrop *et al.*, 1985). The following marker order and recombination frequencies were used in the multipoint sib-pair analysis: D19S391-5%-D19S394-3%-D19S221-5%-D19S226. Affected-sib-pair analysis was performed using the MAPMAKER/SIBS software package, simultaneously including marker information for all four DNA markers (Kruglyak, 1995). Separate analyses were performed for migraine with aura, migraine without aura, and a combination of both. Allowance was made for dominance variance. When more than two affected sibs occurred in a single sibship, weighted scores were computed according to Suarez and Hodge (1979).

In a sib-pair analysis, the occurrence of parental marker alleles is compared among sibs. Normally, 25% of sib pairs share their marker alleles from both parents, 50% share one marker allele from one of their parents, while the remaining 25% share no parental allele. Deviations from this pattern towards increased sharing, and consistent with the constraints of Holmans's (1993) possible triangle, are explained as linkage (expressed as the maximum lod score MLS). Increased sharing of marker alleles thus indicate that the marker is located closely near a genetic risk factor. The relative-risk ratio for a sib (λ_R), defined as the ratio of the prevalence of a disease in sibs of affected individuals, divided by the prevalence of a disease in the population, can be calcutated (May *et al.*, 1995). In other words:

$$\lambda_R = \frac{\text{Affected risk for sib of a proband}}{\text{Affection risk for an individual in the general population}}$$

Results

Genomic structure

The combination of hybridization and PCR strategies resulted in a rapid assembly of the complete coding sequence

of the human cDNA, with an open reading frame of 6783 nucleotides encoding 2261 amino acid residues (figure 4). The spatial distribution of the human Ca^{2+} channel expression was assayed in rhesus monkey tissues. Total RNA was isolated from several tissues, including various brain structures, and probed with a human cDNA fragment. The probe detected a major transcript of approximately 9.8 kb in cerebellum, cerebral cortex, thalamus and hypothalamus, whereas no transcript was detected in heart, kidney, liver or muscle. There was also no hybridization signal found in RNA preparations from mouse skin tissue or from human peripheral lymphocytes. In addition, an attempt to amplify parts of the cDNA from human peripheral lymphocytes failed.

Complete alignment between the cDNA and individual exon sequences was achieved, allowing the establishment of the exon-intron structure (Table 1). The reconstruction of the exon-intron structure of the CACNL1A4 gene revealed 47 exons ranging in size from 36 bp (exon 44) to 810 bp (exon 19). The exons are distributed over about 300 kb at genomic DNA level. The result shows that the first 10 exons are located in a region of about 150 kb covered by the first 5 cosmids of the contig indicating relatively large introns at 5' side of the gene. Sequences (Figure 1) were obtained of all exons including approximately 100 bp of flanking introns, except for intron 5 adjacent to exon 6. The forward primer of exon 6 harbours the splice junction and 3 bp of exon 6. Splice sites around all exons are compatible with consensus sequence with the exception of splice donor and acceptor of the first intron.

The cosmid contig that yielded the initial Ca^{2+} channel gene exons was extended to cover more than 300 kb. Hybridization experiments showed that the first and last cosmids of the contig were positive for 3'- and 5'-end cDNA sequences, respectively, indicating a genomic distribution of the gene over at least 300 kb (Figure 2). The cosmid contig has been placed into the LLNL physical map of chromosome 19 at band p13.1, between the markers D19S221 and D19S226

(Figure 2). We identified a new polymorphic CA-repeat sequence (D19S1150) on the cosmid contig. Oligonucleotide primers (Table 1) flanking the repeat were synthesized and amplification was performed by PCR as described. Analysis of D19S1150 in 45 random individuals from the Dutch population revealed nine alleles with an observed heterozygosity of 0.82. This highly polymorphic marker is located within the gene and is therefore very useful in genetic analysis.

Mutation analysis

Exons and flanking intron sequences, containing the complete coding region of CACNL1A4 and part of untranslated sequences, were screened for the presence of mutations by SSCP and DHPLC analysis in 20 individuals with either FHM or EA-2. Several synonymous nucleotide substitutions and polymorphisms were identified including a highly polymorphic (CAG)n-repeat in the 3' untranslated region of exon 47 (Table 2). Of all polymorphisms only one was identified predicting an amino acid change, an alanine to threonine substitution at codon 454 (A454T).

Four different missense mutations were found in FHM patients of which one mutation was observed in two unrelated FHM affected individuals (Table 3). The mutations were shown to segregate with the disease within the families, and were not present in about 100 control chromosomes. A G-to-A transition was identified in family *Italy-II* at codon 192, resulting in a substitution of arginine to glutamine (R192Q) within the first voltage sensor domain (IS4). A second missense mutation occurs at codon 666, within the P-segment of the second repeat replacing a threonine residue for methione (T666M) in family *USA-P*. Two other mutations are located in the 6th transmembrane spanning segment of respectively repeat II and IV. The IIS6 mutation is a T-to-C transition at codon 714, resulting in a substitution of valine to alanine (V714A), identified in FHM family *UK-B*. The mutation in domain IVS6 is an A-to-C transversion at codon 1811 resulting in a substitution of isoleucine to leucine

(I1811L). This I1811L mutation is found in family *NL-A* and family *USA-C*, two unrelated FHM families. Comparison of haplotypes in this region, including intragenic markers, did not reveal any evidence for a common founder of family *NL-A* and *USA-C* (data not shown). No mutation was found in FHM family *Italy-I* nor in the six sporadic hemiplegic migraine patients. In addition to missense mutations in FHM families, we also identified mutations in two out of four EA-2 families (Table 3). In EA-2 family *CAN-191*, a basepair deletion occurs in exxon 22 at nucleotide position 4073 causing a frameshift and a premature stop. The second EA-2 mutation is a transition of G-to-A of the first nucleotide of intron 24, predicted to leading to an aberrant splicing in family *CAN-26*. The invented gene also contains a CAG repeat, of which expansions have been found in patients with autosomal dominant cerebellar ataxia (SCA6). Hence FHM, EA-2 and SCA6 are allelic ion channel disorders and different mutations are associated with different clinical symptomatologies.

Our study patients with common migraine (with or without aura) included 36 independent multigenerational Dutch families. At least some data were available on 937 family members and 212 persons who "married-in" (spouses). Of these, 442 family members (247 affected) and 86 spouses (7 affected) were personally interviewed. The distribution of the different types of migraine among the 247 affected family members are as follows: 132 family members showed migraine without aura, 93 showed migraine with aura and 22 showed months-migraine, not fulfilling all critera by IHS. Among the 7 affected spouses these figures were 4, 1 and 2, respectively. We scored the parental transmission of migraine in the 36 families (Tabel 4) to investigate if an additional X-linked dominant or mitachondrial gene was involved. An approximately 2.5:1 preponderance of females among the migraine sufferers was noted, which remained in the affected offspring. Affected fathers were found to transmit migraine

to their sons in 21 cases, making X-linked dominant or mitochondrial inheritance highly unlikely.

The genetic analysis included 204 potentially affected sib pairs; after correction for more than one sib pair in a single sibship the total number of sib pairs was 108. Affected-sib-pair analysis was performed for sib pairs who were both affected with any form of migraine and, in separate analyses, for sib pairs who where both suffering from either migraine with aura or migraine without aura. The informativeness of the region between the markers D19S391, D19S394, D19S221 and D19S226 varied between 82% and 96%. The combined analysis of migraine with and without aura resulted in a maximum multipoint lod score of 1.69 ($p \approx 0.005$) with marker D19S226. For migraine with aura the maximum multipoint lod score was 1.29 corresponding with $p \approx 0.013$ with marker D19S394. The maximum lod score for migraine without aura was not significant (MLS <0.25)(data not shown). The relative risk ratio for a sib to suffer from migraine with aura (λ_R), defined as the increase in risk of the trait attributable to the 19p13 locus, varied between $\lambda_R=1.5$ (for marker D19S394) and $\lambda_R=2.4$ (for marker D19S226). When combining migraine with and without aura, λ_R was 1.25. In a selected portion of 36 Dutch families with migraine with aura and without aura, affected sib-pair analysis was performed for sib pairs who were affected with any form of migraine. The following markers, flanking the β2(CACNB2) calcium channel subunit gene on chromosome 10p12, were tested: D108191, D10S246 and D10S89. For the combined phenotype (migraine with and without aura) a maximum pultipoint iod score of 0,95 ($p<0,01$) was obtained with marker; D10S191. This result gives independent evidence for a role of the P/Q type Ca^{2+} channel in migraine and other neurological disorders.

Discussion

The genomic structure of the exemplified invented gene revealed 47 exons distributed over about 300 kb (Table 1; Figure 1). A comparison of the gene structure to already

known Ca^{2+} channel α1 subunit genes (CACNL1A1, CACNL1A2, and CACNL1A3) (Soldatov, 1994; Yamada *et al.*, 1995; Hogan *et al.*, 1995), reveals a similar number of exons (50, 49, and 44 respectively) but a larger genomic span (300 kb vs 90-150 kb). Remarkebly, all splice sites are according to consensus sequence except for intron 1. Splice donor as well as splice acceptor of the first intron do not contain the expected gt...ag intron sequence. An incorrect cDNA sequence is unlikely because the cDNA sequence containing the junction of the first two exons is identical to rabbit and rat sequence. Sequences corresponding to splice donor and acceptor are present in exon 1 and 2, suggesting an additional (yet unidentified) exon in the first intron encompassing part of sequences of exon 1 and exon 2.

To test the possible involvement of the invented gene relating to the CA^{2+}-channel sub-unit in migraine FHM, SCA6 and EA-2, we performed a mutation analysis by DHPLC and SSCP and found several alterations (For example Table 2 & 3). Only one missense variation was observed also present in 2% of the normal controls (Table 2). This polymorphism is a alanine to threonine substitution at codon 454 (A454T), located in the intracellular loop between IS6 and IIS1 (Figure 2). This region contains a conserved alpha interaction domain (AID) that binds subunits (De Waard *et al.*, 1996). However, A454T is located outside the AID consensus sequence and is not likely to be involved.

The identification of two mutations that disrupt the predicted translation product of the invented gene in two unrelated EA-2 patients and the segregation of these mutations with the episodic ataxia phenotype in their families provide strong evidence that the invented gene is the EA-2 gene. A basepair deletion leads to a frame-shift in the putative translation product and encounters a stop codon in the next exon. The frame-shift in this EA-2 family is predicted to yield a calcium channel α1 subunit polypeptide consisting of repeat I and II, and a small portion of repeat III (IIIS1). The G-to-A transition of the first nucleotide of

intron 24 is affecting the nearly invariant GT dinucleotide of the intronic 5' splice junction. The brain-specific expression of the exemplified invented gene makes it extremely difficult to test the hypothesis that this mutation produces aberrantly spliced RNAs by retaining the intron or utilizing other cryptic 5' splice sites.

The frameshift and splice site mutations in EA-2 may suggest a dominant negative effect of the truncated proteins by overruling the (corresponding) intact α1 subunits.

No mutations were found in the remaining EA-2 families (CAN-25 and -197). The use of two independent techniques for mutation screening (DHPLC and SSCP) makes it unlikely that we missed a heterozygote PCR product. Mutations in the promoter region or in intron sequences, resulting in aberrant splicing, may have been the cause of EA-2 in these families. We could also have missed a mutation around the splice acceptor site of intron 5, covered by the forward primer of exon 6. However, larger deletions of *e.g.* complete exons with flanking intron sequence will disturb the predicted translation product, like the ΔC_{4073} and splice site mutation do, but this is not detectable by a PCR-based screening method but can be seen Southern blot analysis instead.

Four different missense mutations were identified in five unrelated FHM families. These mutations all segregate with FHM within a family and are not observed in over 100 normal chromosomes. The first missense mutation that we describe in the exemplified invented gene occurs in the IS4 domain of the α1 subunit (Table 3; Figure 3). The S4 domains are postulated to be voltage sensors because they have an unusual pattern of positively charged residues at every third or fourth position separated by hydrophobic residues (Tanabe *et al.*, 1987). In calcium channels the positively charged amino acid is an arginine residue (Stea *et al.*, 1995). The mutation in FHM family *Italy-II* predicts a substitution of the first arginine in the IS4 segment with a neutral, non-polar glutamine (R192Q). The change of the net positive

charge of this conserved region of the protein may influence correct functioning of the voltage sensor.

The second missense mutation in FHM family *USA-P* occurs in the P-segment of the second transmembrane repeat. A C-to-T transition predicts substitution of a threonine residue with methionine at codon 666 (T666M). Various observations have shown that P-segments, the hairpin between S5 and S6 that spans only the outer part of the transmembrane region, form the ion-selectivity filter of the pore and binding sites for toxins (Guy and Durell (1996). Alignment of protein sequence of different P-segments indicating that some residues occur in many different channel genes (Guy and Durell, 1996). The T666M substitution alters one of the conserved residues in the P-segment. It is conceivable that an alteration of a P-segment affects the ion-selectivity or toxin binding of a channel gene.

The remaining two missense mutations identified in FHM families alter the S6 segment of the second and the fourth repeat. A valine to alanine substitution in FHM family *UK-B* is found in domain IIS6 at codon 714 (V714A). Domain IVS6 is mutated in two unrelated FHM families (*NL-A* and *USA-C*), predicting a isoleucine to leucine substitution at codon 1811 (I1811L). The V714A and I1811L missense mutations do not really change the neutral-polar nature of the amino acid residues. However, both S6 mutations are located nearly at the same residue at the intracellular site of the segment and are conserved in all calcium channel α1 subunit genes. In addition, the A-to-C transversion leading the I1811L substitution occurred in two unrelated FHM families on different haplotypes indicating recurrent mutations rather than a founder effect. Although the exact function of the S6 domains are not known, these data strongly suggest that mutations in IIS6 and IVS6 result in FHM.

The I1811L mutation is present in two FHM families of which one (*NL-A*) also displays a cerebellar atrophy in (some) affected family members. The presence of cerebellar atrophy in FHM families has been reported in about 40% of chromosome

19-linked FHM families, whereas none of the unlinked families was found to have cerebellar atrophy (Terwindt *et al.*, 1996).

The I1811L mutation excludes the possibility of allelic mutations in FHM and FHM with cerebellar atrophy. However, it is likely that FHM or FHM with cerebellar atrophy are the result of pleiotropic expression of a single defective gene.

No mutation was found in a small Italian FHM family (*Italy-I*). Apart from the possibilities discussed for EA-2, it should be noted that linkage to 19p13 was only suggested but never proved with significant lod scores (M. Ferrari, personal knowledge).

The four missense mutations identified indicate a mechanism for FHM in which both alleles of the α1 subunit are expressed, one harbouring an amino acid substitution which affects the function of this calcium channel α1 subunit by reducing or enhancing the electrical excitability. The relationship of FHM and other types of migraine makes it highly rewarding to investigate the involvement of the only missense variant observed (A454T) (Table 2), and tó continue the search for other variants of the exemplified invented gene specific for common types of migraine.

The mutations in EA-2 and FHM demonstrate among others that the brain specific calcium channel gene CACNL1A4 is responsible for both EA-2 and FHM, and is also involved in the primary pathogenesis of the more common forms of migraine. We conducted the common migraine study in an independent sample of 36 extended Dutch families, with migraine with aura and migraine without aura. We found significant increased sharing of the marker alleles in sibs with migraine with aura (MLS=1.29 corresponding with $p \approx 0.013$). Although no such increased sharing was found for migraine without aura, a combined analysis for both migraine types resulted in an even more significant increased sharing (MLS=1.69 corresponding with $p \approx 0.005$). These resúlts clearly indicate the involvement of the calcium α_{1A}-subunit gene region on 19p13 in both migraine with and without aura; the contribution to migraine with aura, however, seems strongest.

The positive findings in our study clearly demonstrate an involvement of the FHM locus region in non-hemiplegic familial migraine, notably in migraine with aura. The P/Q-type calcium channel α_{1A}-subunit gene on chromosome 19p13 may be an "aura-gene" and is involved in both FHM and migraine with aura, but not in migraine without aura. This however, seems unlikely since an increased sharing of marker alleles was also found when we combined the results for migraine with and without aura. Furthermore, the increase in sharing was stronger than expected to be only due to the contribution of migraine with aura. An alternative explanation is that the gene is involved in both types of migraine, but in migraine without aura there is an additional strong effect of other, possibly environmental factors, thereby reducing the penetrance.

The latter view is also supported by the results obtained from calculating the relative risk ratios (λ_R) for sibs from affected individuals to also have migraine. The relative risk ratio for a sib to suffer from migraine with aura was λ_R=2.4. When combining migraine with and without aura, λ_R was 1.25. In a population-based study the relative risk for first degree relatives of probands with migraine with aura to also have migraine with aura was λ_R=3.8. Because of the female preponderance among migraine patients, X-linked dominant or mitochondrial inheritance has been suggested to be involved in familial migraine. Although a predominant maternal inheritance pattern was noted in our families, X-linked dominant or mitochondrial inheritance were found to be highly unlikely because affected fathers transmit migraine to their sons. Furthermore, the predominant maternal inheritance can be explained by the female preponderance among the migraine patients.

We conclude that the well-established genetic contribution to the etiology of migraine is partly, but not entirely, due to genetic factors located in the chromosomal region of the P/Q-type calcium channel α_{1A}-subunit gene. Further analysis of the cerebral distribution and function of

this calcium channel, as well as of the "mutated channels", will help to unravel the pathogenetic pathway of migraine. It may also contribute to a better understanding of the mechanisms involved in related disorders such as episodic ataxia type-2, autosomal dominant cerebellar ataxia (SCA6), cerebral atrophy, and epilepsy, which all have been found to be associated with mutations in this gene. Study of FHM, EA-2 mutants and variants such as the A454T variant expressed in vitro or in mouse or other experimental animal models will ultimately lead to better understanding of the diseases, their cellular mechanisms, and the clinical relationship between FHM, EA-2, migraine, and other episodic neurological disorders such as epilepsy, and will provide rationales for the development of prophylactic therapy.

Localization and identification of the mouse gene related to the neurological mouse mutations tottering, leaning and rolling.

The tottering (tg) mutation arose spontaneously in the DBA inbred strain, and has been back-crossed into a C57BL/6J (B6) inbred strain for at least 30 generations. The genome of the tg mouse therefore is of B6 origin except for a small region around the tg gene on chromosome 8. Interestingly, the chromosome 8 region in mouse has synteny with the human chromosome 19p13.1, in which the human calcium channel alpha1 subunit has been identified. We therefore consider the tg locus as a possible site of the mouse homologue of the human calcium channel gene.

To determine the exact localization of the mouse homologue, PCR was carried out with primers based on human cDNA sequence selected from Figure 1 and mouse genomic DNA as template. In human, primers were known to be located in different flanking exons. PCR amplification on human DNA yielded a 1.5kb fragment.

Forward primer : 5'- caa cat cat gct ttc ctg cc-3'
Reversed primer: 5'- atg atg acg gcg aca aag ag-3'

Amplification on mouse DNA yielded a 750-bp fragment. The fragment mainly consists of intronic sequences. SSCP analysis revealed several polymorphisms in the different inbred strains (each strain a specific pattern). Analysis of amplified product of the tg/tg (homozygote) and tg/+ (heterozygote) mice demonstrated a DBA specific signal in the tg/tg mouse, and a heterozygous pattern of DBA and B6 inbred strains in the heterozygous tg/+ mouse.
These results show that the mouse homologue of the human calcium channel alpha1 subunit is located within the mouse tottering interval on chromosome 8.

In conclusion: the phenotypic characteristics of the tg mouse (tg/tg and tg/+) suggest involvement of ion-channels in the tg-etiology. The localization of the mouse homologue of the human calcium gene within the tottering interval show that a tottering phenotype in mouse is caused by a mutation in the mouse homologue of the CACNL1A4 gene. With various variants of the tottering mouse (the Jackson Laboratory, Bar Habor, ME, USA), such as the leaner and rolling varieties, such mutations in the mouse homologue of the CACNL1A4 gene can be found, clearly demonstrating that the gene is related to a variety of episodic neurologic disorders and using this genetic information one can engage in a variety of pathofysiological studies, as for example indicated below.

The tg mutation arose spontaneously in the DBA/2 inbred strain. tg/tg homozygotes are characterized by a wobbly gait affecting the hindquarters in particular, which begins at about 3 to 4 weeks of age, and by intermittent spontaneous seizures which resemble human epileptic absence seizures. The central nervous system of the mice appears normal by light microscopy. There is no discernible cerebellar hypoplasia. In fluorescent histochemistry studies tg/tg mice show a marked increase in number of noradrenergic fibers in the terminal fields innervated by locus ceruleus axons, the hippocampus, cerebellum, and dorsal lateral geniculate. Treatment of neonatal tg/tg mice with 6-hydroxydopamine, which selectively causes degeneration of distal noradrenergic axons from the

locus ceruleus, almost completely abolishes the ataxic and seizure symptoms.

The leaner mutation of the tottering mouse arose spontaneously in the AKR/J strain. Homozygotes are recognized at 8 to 10 days of age by ataxia, stiffness, and retarded motor activity. Adults are characterized by instability of the trunk, and hypertonia of trunk and limb muscles. The cerebellum is reduced in size, particularly in the anterior region, in tg<la>/tg<la> mice, as is the case with a certaim number of FHM patients. There is loss of granule cells beginning at 10 days of age and loss of Purkinje and Golgi cells beginning after 1 month. Cell loss later slows but continues throughout life. Granule and Purkinje cells are more severely affected than Golgi cells and the anterior folia more severely affected than other parts of the cerebellum. The cerebellum of tg<la>/tg mice shows shrinkage and degenerative changes of the Purkinje cells. The loss in cerebellar volume in tg<la>/tg and in tg/tg mice is specific to the molecular layer, with no change in volume of the granule cell layer or the white matter layer. Allelism of leaner with tottering was shown in complementation and linkage tests.

A third variety of the tottering mouse is (tg<rol>) called the rolling Nagoya. Found among descendants of a cross between the SIII and C57BL/6 strains, the tg<rol> mutation apparently occurred in the SIII strain. Homozygotes show poor motor coordination of hindlimbs that may lead to falling and rolling, and sometimes show stiffness of the hindlimbs and tail. No seizures have been observed. Symptoms are recognizable at 10 to 14 days old. They appear a little earlier than those of tg/tg mice and are somewhat more severe. The cerebellum is grossly normal until 10 days of age, but after that grows more slowly than normal. The size of the anterior part of the central lobe of the cerebellum is reduced with reduction in the numbers of granule, basket, and stellate cells but normal numbers of Purkinje cells. There is a reduced concentration of glutamate and an increasd

concentration of glycine and taurine in the cerebellum and decreased activity of tyrosine hydroxylase in the cerebellum and other areas.

Legends to figures

Figure 1
Nucleic acid sequences of 47 exons and flanking intron sequences containing the complete coding region of the invented gene and part of untranslated sequences.

Figure 2
Genetic map, cosmid contig and global exon distribution of the invented gene om chromosome 19p13.1. The cosmid contog is shown with EcoRI restriction sites, available via Lawrence Livermore National Laboratory; exon positions are indicated schematically, regardless of exon or intron sizes (Table 1). D19S1150 is a highly polymorpmic intragenic (Ca)n-repeat.

Figure 3
Membrane topology of α1 subunit of the P/Q-type Ca^{2+}-channel. The location and amino acid substitutions are indicated for mutations that cause FHM or EA-2

Figure 4
The coding sequence of human cDNA of the invented gene with an open reading frame encoding 2261 amino acid residues.

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Exon/intron organization of the human invented gene
and exon-specific primer pairs

Table 1

Exon	cDNA	Size	Domain	Cosmid(s)	Primer Forward	Primer Reversed	Size
1	UTR - 568	±500		25960 / 30151	tct ccg cag tcg tag ctc ca cgc aaa gga tgt aca agc ag	ggt tgt aga gtg cca tgg tc att ccc aag cct cca ggg tag	320 370
2	569 - 674	106	I S1	30151	cac ctc caa cac cct tct tt	tct gtg ccc tgc tcc act c	240
3	675 - 814	140	I S2, I S3	30151	acg ctg acc ttg cct tct ct	caa cca aaa gcc tcg taa tc	230
4	815 - 906	92	I S3, I S4	28913	aaa acc cac cct ctg ttc tc	ttg tca ggg tcg gaa act ca	160
5	907 - 1059	153	I S5	28913 / 27415	ctt ggt ggc ggg gtt t	ctg cct aat cct ccc aag ag	290
6	1060 - 1253	194		27415	tcc ctt ccc ttt tgt aga tg	gtg ggg ctg tgt tgt cct t	350
7	1254 - 1357	104	I S6	27415	gac aga gcc aca aga gaa cc	agc aaa gag gag tga gtg gg	250
8	1358 - 1473	116		34077 / 27415	ata ctc tgg ctt ttc tat gc	gca tga ctc tct ttg tac tc	230
9	1474 - 1530	57		34077	gca gag aat ggg ggt gg	ctg agg tgg gtt tag agc ag	180
10	1531 - 1623	93		34077	ggg taa cgt ctt ttt ctc ttg c	atg tct ctt ggg cga tag gt	200
11	1624 - 1833	210	II S1	16894 / 32236	att tct tct gaa gga aca gc	gga ggg atc agg gag ttg gc	310
12	1834 - 1946	113	II S2, II S3	16894	caa gcc taa cct cct ctc tg	tca ttc cag gca aga gct g	200
13	1947 - 2051	105	II S3, II S4	16894	att tgg agg gag gag ttt gg	tca ctt tcc caa ctt tct gg	310
14	2052 - 2191	140	II S4, II S5	16894	cag aaa gtt ggg aaa gta gc	ttg aat tcc tgt gaa gga c	250
15	2192 - 2264	73		16894	ctt gga gat gag ata ctg agc	cag gca ctt tca tct gtg ac	200
16	2265 - 2382	118	II S6	16894	tcc aca gct gca tct cca ag	acc ctc cct tga gcc cct	270
17	2383 - 2450	68	II S6	16894	cag tgg ttg ctt ttc ctg ac	ttg cca gag aaa cat tct cc	130
18	2451 - 2557	107		16894	tga aca aag att cca cgt gc	ttc agg agc cag ggt agc atc	170
19	2558 - 3367	810		16894	tag caa tgc tct aag tcc tg cgc agg aga acc gca aca a gc agc agg gag agc cgc agc	tgt ttc ctg agg aag tcc tc gcg atg acg tcg atg ctc tac cgt cat tct gcg gat tc	320 450 300
20	3368 - 3831	464		16894	ggt tct ttt tca ttc act tgc gag aat agc ctt atc gtc ac	ttt cct ggc agt ctt agc tg cag tga tgt gag agc aga g	430 200
21	3832 - 3973	142		16894 / 34275	tgg gaa att gtg gag gga gc	tga ctt ccg cca ccc tgg tg	250
22	3974 - 4103	130	III S1	16894 / 34275	agc ctg tgg tct gag tgg ac	tag gaa ggg gtg tgc tct gtg	210
23	4104 - 4163	60	III S2, III S3	16894 / 34275	atc cac tgc tct ctt gct tt	gtg gtt ctc act tat aat ctg c	170
24	4164 - 4270	107	III S3	34275	tgg cct cat tgg ctt ccc tgc	aag agg aaa ccc ttg cga ag	250
25	4271 - 4370	100	III S4	34275	cta ccc aac ctg acc tct gc	aca tga taa ccc tga cag tc	220
26	4371 - 4531	161	III S5	34275	ctc atg ctc tct gtc aac tc	tgg ttc caa tgg gaa tgt gc	250
27	4532 - 4669	138		34275	ctg ctt ccc aag cag tct ag	tcc tgg ata gat ttc cag tc	300
28	4670 - 4871	202	III S6	34275	agt ttt taa agg aca gat gg	ttt ccc tgc ccc att cct ttg c	280
29	4872 - 5036	165	IV S1	34275	ctc tgc cgc tct cac cac tg	ttt atc agg tag agg cag g	250
30	5037 - 5147	111	IV S1, IV S2	34275	ttc caa gcc cat agc tgt agc	tga ccc tgc tac tcc tgc ttc	180

31	5148 - 5231	84	IV S3	15496	act gtg cct cta aca tgc ac	aag tgc tgg ctc aag cag	250
32	5232 - 5348	117	IV S4	15496	tct gtg agt ggt gac agc tc	gtc acc tgt ctt ctc agc	240
33	5349 - 5414	66	IV S5	15496	tgg aag gac tct ggc acg tg	gga ggc tct ggg aac ctt ag	250
34	5415 - 5530	116		15496	aga agc cac tgg agg aat ggc	att atc aga gca ggt ccc ctt c	250
35	5531 - 5681	151	IV S6	15496	tcc gag tct ctg att tct cc	aga cgg ccc tca cag tgt c	210
36	5682 - 5809	128	IV S6	15496	ttc att ccc tcg gtc tct gc	ctg act gaa cct gtg aga c	350
37	5810 - 5906	97		15496	tgt gaa ccc att gcc tgc a	tgg gaa tga ctg cgc ttg c	200
38	5907 - 6012	106		15496	atg cct ggg aat gac tgc	tgt cac gcc tgt ctg tgc	200
39	6013 - 6120	108		15496	tga cac cca ggc agg cag	tct gtc ctg gtg gat tgg atc	200
40	6121 - 6221	101		15496	ttg gtg agc tca ccg tgt	ttc ccg tgg tga cat gca agc	200
41	6222 - 6331	110		15496	gtc cac aca ctg ctc tct gc	aca ctc cac ctc cct ggc	320
42	6332 - 6470	139		15496	gcc agg gag gtg gag tgt	ggt tcc ttc cac cgc aac	550
43	6471 - 6584	114		15496 / 30762	caa ctc ccc aat ggc tc	cct acc cag tgc aga gtg agg	350
44	6585 - 6620	36		15496 / 30762	tct gtg tgc acc atc cca tg	aag gat tgg gct cca tgg ag	200
45	6621 - 6807	187		15496 / 30762	gtt ggt gct agc tgc tga c	ctt tct tct tcc tta gtg tc	330
46	6808 - 7061	254		15496 / 30762	gtg tgc tgt ctg acc ctc ac	agc ctg ggg tca ctt gca gc	320
47	7062 - UTR	≥350		/ 30762	cct ttg ttt caa ttt tcg tgt ag	tgg ggc ctg ggt acc tcc aa	280

Note. Sizes of exons and PCR products are given in basepairs; domains of protein are indicated according to Stea et al., 1995.

Table 2 Polymorphisms in coding sequence of the invented gene

Location	*Nucleotide change*			*Frequency*	*Consequence*
exon 4	nt 854	G - A	Thr_{193}	0.02	-
exon 6	nt 1151	A - G	Glu_{292}	0.07	-
exon 8	nt 1457	G - A	Glu_{394}	0.38	-
exon 11	nt 1635	G - A	Ala_{454}	0.02	Ala_{454} - Thr (A454T)
exon 16	nt 2369	G - A	Thr_{698}	0.12	-
exon 19	nt 3029	G - A	Glu_{918}	0.07	-
exon 23	nt 4142	T - C	Phe_{1289}	0.22	-
exon 46	nt 6938	T - C	His_{2221}	0.46	-
exon 47	nt 7213	$(CAG)_n$	3'UTR	#	-

Note. Frequency as observed in 100 control chromosomes: # Seven alleles of $(CAG)_n$ were observed in the range between n=4 to n=14, with a heterozygosity value of 0.75.

Table 3 Mutations of the invented gene in families with FHM or EA-2

Disease	Family	Location	Domain	Nucleotide change		Consequence	
FHM	It-II	exon 4	I S4	nt 850	G - A	Arg_{192} - Gln (gain of SfcI site)	R192Q
FHM	US-P	exon 16	P-segment	nt 2272	C - T	Thr_{666} - Met	T666M
FHM	UK-B	exon 17	II S6	nt 2416	T - C	Val_{714} - Ala (gain of BbvI site)	V714A
FHM	NL-A/US-C	exon 36	IV S6	nt 5706	A - C	Ile_{1811} - Leu (gain of MnlI site)	I1811L
EA-2	CAN-191	exon 22	III S1	nt 4073	deletion C	frameshift (loss of NlaIV site)	$STOP_{1294}$
EA-2	CAN-26	intron 24	splice site	nt 4270-1	G - A	AC/gt - AC/at (loss of BsaAI site)	aberrant splicing

Table 4. **Parental transmission of migraine for 36 unrelated Dutch families.**

parents	*N*	*offspring*	*N*	*affected N(%)*	*ratio**
heathy father x migraine mother	51	daughters	72	48 (66.7%)	2.3:1
		sons	72	21 (29.2%)	
migraine father x healthy mother		daughters	26	17 (65.4%)	2.5:1
	18	sons	15	4 (26.7%)	

* ratio of proportion affected sons/proportion affected daughters

CLAIMS

1. An isolated and/or recombinant nucleic acid encoding a Ca^{2+}-channel α1 subunit related to (familial hemiplegic) migraine and/or episodic ataxia type-2 derived from, related to or associated with a gene which in humans is present on chromosome 19p13.1-19p13.2 or a specific fragment or homolog or derivative thereof.

2. A nucleic acid according to claim 1 which is a cDNA molecule.

3. A cDNA molecule according to claim 2 comprising a 6800 bp coding region.

4. A nucleic acid according to claim 1, 2 or 3 which is of human origin.

5. A nucleic acid according to claim 4 and showing at least 70% homology with the nucleic acid sequence as listed in figure 1.

6. A nucleic acid according to any of claims 1-5 and showing at least 90% homology with the nucleic acid sequence as listed in figure 1.

7. A nucleic acid according to any of claims 1-6 and showing a mutation at codon 192 resulting in the replacement of arginine by glutamine.

8. A nucleic acid according to any of claims 1-7 and showing a mutation at codon 666 resulting in the replacement of threonine by methionine.

9. A nucleic acid according to any of claims 1-8 and showing a mutation at codon 714 resulting in the replacement of valine by alanine.

10. A nucleic acid according to any of claims 1-9 and showing a mutation at codon 1811 resulting in the replacement of isoleucine by leucine.

11. A nucleic acid according to any of claims 1-10 and comprising a CA-repeat sequence as shown in figure 2.

12. A nucleic acid according to any of claims 1-11 and comprising a (CAG)n repeat sequence as shown in figure 2.

13. A nucleic acid according to any of claims 1-12 and comprising a polymorphism in the coding sequence.

14. A nucleic acid according to claim 13 and comprising a polymorphism in the coding sequence as shown in table 2.

15. A nucleic acid according to claim 13 or 14 and comprising a mutation at codon 454 resulting in a replacement of alanine by threonine.

16. A nucleic acid according to any of claims 1-15 and comprising a deletion.

17. A nucleic acid according to any of claims 1-16 and comprising a frameshift at codon 1266.

18. A nucleic acid according to any of claims 1-17 and comprising a mutation resulting in abberant splicing.

19. A nucleic acid according to any of claims 1-18 and comprising a mutation resulting in abberant splicing of intron 28.

20. An isolated and/or recombinant nucleic acid encoding a CA^{2+} channel subunit or a functional fragment thereof related to (familial hemiplegic) migraine and/or episodic ataxia type 2.

21. An isolated and/or recombinant nucleic acid encoding a CA^{2+} channel β2 subunit related to (familial hemiplegic) migraine and/or episodic ataxia type 2, derived from, related to or associated with a gene which in humans is present on chromosome 10p12 or a specific fragment thereof.

22. A method for localising or identifying a gene using a nucleic acid molecule or a fragment of fragments thereof according to any of claims 1-21.

23. A method according to claim 22 wherein the gene is related to episodic neurological disorders.

24. A method according to claim 22 or 23 wherein the gene is related to migraine.

25. A method according to claim 22, 23 or 24 wherein the gene is related to FHM and/or EA-2 and/or autosomal dominant cerebellar ataxia.

26. A method distinguishing between alleles of a gene using a nucleic acid molecule or a fragment of fragments thereof according to any of claims 1-21.

27. A method according to any of claims 23-26 in which the gene is of human origin.

28. A method according to any of claims 23-27 which comprises selecting a cell or an animal.

29. A recombinant expression vector comprising a nucleic acid molecule or a fragment of fragments thereof according to any of claims 1-21.

30. A cell or an animal comprising a vector according to claim 29.

31. A cell or an animal comprising a nucleic acid molecule or a fragment of fragments thereof according to any of claims 1-21.

32. A cell or an animal selected by a method according to claim 28.

33. A cell or an animal comprising a genome in which nucleic acid sequences corresponding to nucleic acid molecules according to any of claims 1-21 have been modified.

34. Use of a cell or an animal according to any of claim 30-33 to test or develop specific medication for the treatment of FHM, EA-2, SCA6, migraine or other neurological disorders associated with cation channel dysfunction.

35. A protein or peptide comprising an amino acid sequence encoded by a nucleic acid molecule, or a fragment or fragments thereof, according to any of claims 1-21.

36. A natural or synthetic antibody directed against a protein or peptide according to claim 35.

37. Use of a protein or peptide or antibody according to claim 35 or 36 to detect or diagnose FHM, EA-2, SCA6, migraine or other neurological disorders associated with cation channel dysfunction.

Figure 1

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//
Submission no   :    1
exon 1          :    <..672
start codon     :    381..383
intron 1        :    673.
Remarks         :    no consensus splice site intron 1

ttttttttacg ttctcttttt tttcgagtgg tgactggatg ctgattcttc    50
ctcgtatttt tgctgcttct ctctccctcc cctccttccc gggcccgggc   100
ccgccccgca ccctccttcc gcccctcctt ctccggggtc agccaggaag   150
atgtcccgag ctgctatccc cggctcggcc cgggcagccg ccttctgagc   200
ccccgacccg agcgccgagc cgccgcgcga tgggctgggc cgtggagcgt   250
ctccgcagtc gtagctccag ccgccgcgct cccagccccg gcagcctcag   300
catcagcggc ggcggcggcg gcggcggcgt cttccgcatc gttcgccgca   350
gcgtaaccgg agccctttgc tctttgcaga ATGGCCCGCT TCGGAGACGA   400
GATGCCGGCC CGCTACGGGG GAGGAGGCTC CGGGGCAGCC GCCGGGGTGG   450
TCGTGGGCAG CGGAGGCGGG CGAGGAGCCG GGGGCAGCCG GCAGGGCGGG   500
CAGCCCGGGG CGCAAAGGAT GTACAAGCAG TCAATGGCGC AGAGAGCGCG   550
GACCATGGCA CTCTACAACC CCATCCCCGT CCGACAGAAC TGCCTCACGG   600
TTAACCGGTC TCTCTTCCTC TTCAGCGAAG ACAACGTGGT GAGAAAATAC   650
GCCAAAAGAT CACCGAATGG CCatatcctt ttgcccgaac cccagcagca   700
gctgcgcctc cccctcctcc ctccgcctcc cctcttccag gctgggagag   750
agacccgggg gttgatggga ggtggggagg agggggtct tccaggggct   800
gggagagggg gcaccgggag gagtgtgaaa gaatctctcc accccgagct   850
gggttgagct accctggagg cttgggaatg ggtttttcgg gggctggggg   900
ccggccagcc ggagagtgga tccttcccaa ggaccgactc tagaatgaga   950
tct                                                      953
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//
Submission no     :    2
Intron 1          :    <..88
Exon 2            :    89..194
Intron 2          :    195..>
Remarks           :    No consensus splice site intron 1

gatctttycc actggggtca gtgggggtgg gtgcacctcc aacacccttc     50
ttttctttga acaagatttt tccttaattc cccaatacTC CCTTTGAATA    100
TATGATTTTA GCCACCATCA TAGCGAATTG CATCGTCCTC GCACTGGAGC    150
AGCATCTGCC TGATGATGAC AAGACCCCGA TGTCTGAACG GCTGgtgagt    200
gatgtctttt ctcagggtct tctccttggc tttagcagga cattaatttt    250
tgggggagtg gagcagggca cagaggaggc tctcagtcct ggagcccaga    300
gccagatcat gggaagccta aatttccttt tcattttttc ttgaaccaga    350
gtctcgctct gtcacccagg ctggagtgca gtggttcagt catagctcac    400
tgcagcctcc acctcctggg ctcaagccat cctcccactg cagcctcctg    450
agtagcaggg actaacaggt gccaccatgc ccagttaatt ttcttatttt    500
tatctttttt tgtaagaaga tggggat                              527
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//
Submission no   :     3
Intron 2        :     <..57
Exon 3          :     58..197
Intron 3        :     198..>

     gatcttgtca acatctgccc agcccaagac gctgaccttg ccttctctcc      50
     cttccagGAT GACACAGAAC CATACTTCAT TGGAATTTTT TGTTTCGAGG     100
     CTGGAATTAA AATCATTGCC CTTGGGTTTG CCTTCCACAA AGGCTCCTAC     150
     TTGAGGAATG GCTGGAATGT CATGGACTTT GTGGTGGTGC TAACGGGgta     200
     agtggcgcgt gctatacgct ttggatttaa ctagctgaag gattacgagg     250
     cttttggttg gtgtggtccg ggccaggctc aggaaggctg agcccttgtg     300
     ttctccctcc ccttgttatg cgcctgcctc ctttctgcca acacccccacc    350
     tccatgtctc agctgtatat tacagcagat gctttctgtt acaattaaaa     400
     taatagctca ttattgttgg ctgcttccag agtgctttat g              441
```

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//
Submission no  :    4
Intron 3       :    <..142
Exon 4         :    143..234
Intron 5       :    235..>

     aaaactgagg ccagtggtgt cgagtcacct gcctgtggtc acccaaccaa        50
     tacaggacag cttggaatcc caagcacccc cgccctgctg tctgaccccc       100
     aaaacccacc ctctgttctc cattctggct tctttctttc agCATCTTGG       150
     CGACAGTTGG GACGGAGTTT GACCTACGGA CGCTGAGGGC AGTTCGAGTG       200
     CTGCGGCCGC TCAAGCTGGT GTCTGGAATC CCAAgtgcgt gagtttccga       250
     ccctgacaa                                                    259
```

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//
Submission no   :     5
Intron 4        :     <..118
Exon 5          :     119..271
Intron 5        :     272..>

cttaatattc cctcaggaac acacctgctt tgtctgggag agacctgggc    50
gtcttggtgg cggggttttg ggggtacttg ctcatgggct tatggggcct   100
ctctctgtgt ccccccagGT TTACAAGTCG TCCTGAAGTC GATCATGAAG   150
GCGATGATCC CTTTGCTGCA GATCGGCCTC CTCCTATTTT TTGCAATCCT   200
TATTTTTGCA ATCATAGGGT TAGAATTTTA TATGGGAAAA TTTCATACCA   250
CCTGCTTTGA AGAGGGGACA Ggtaggtcca cggagcatga tgcatctttc   300
cagttttctc cttcagggac aagctcttgg gaggattagg caggggtgtg   350
cttctttctc ctggcagctg ggaggaccgt ctccttcaga gagcactac    399
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//
Submission no    :    6
Intron 5         :    <..22
Exon 6           :    23..216
Intron 6         :    217..>

tttttcccct tccctttgt agATGACATT CAGGGTGAGT CTCCGGCTCC  50
ATGTGGGACA GAAGAGCCCG CCCGCACCTG CCCCAATGGG ACCAAATGTC 100
AGCCCTACTG GGAAGGGCCC AACAACGGGA TCACTCAGTT CGACAACATC 150
CTGTTTGCAG TGCTGACTGT TTTCCAGTGC ATAACCATGG AAGGGTGGAC 200
TGATCTCCTC TACAATgtaa gtgatgctgg gacagtgtgt gtggacaatc 250
agagtctcag ggaggtggcc tcctgggacc agtgagactc caaggctgca 300
atggagggac cctgagctgg gaaaggcagc ccaaggacaa cacagcccca 350
ctgaagctgg cctgaggctc aggcttttga agattacagg ggctcatgag 400
cagaactcta actatagggc atagaagtct ggagggcccc cagatgcaac 450
atcatttttc attgtgcaag tgtttagata taattttaga tttttgaata 500
cggaaaggtt atgtgatcca aaatccaaca cagataaaag atagagtaat 550
atctttggac gtaggcgagg ggtccctgcc ctgagg                586
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Submission no   :     7
Intron 6        :     <..183
Exon 7          :     184..287
Intron 7        :     288..>

tttcttcaga aaacggttcc ttcctccatt tccccctctg ggatgccaga     50
gccccagaac tccacaagcc aagaacattt aagacagagc cacaagagaa    100
ccgagcttcc ccttccctca cctgtcaggt tctatctgag tcccagtcaa    150
ctctcacctg ctttccctcc tcacacccta cagAGCAACG ATGCCTCAGG    200
GAACACTTGG AACTGGTTGT ACTTCATCCC CCTCATCATC ATCGGCTCCT    250
TTTTTATGCT GAACCTTGTG CTGGGTGTGC TGTCAGGgta agtttctgct    300
actccccacc ccatcccact cactcctctt tgctaacttc tttccaagta    350
gaggccattg aagctttgtt ttcattcact agacaga                  387
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//
Submission no  :    8
Intron 7       :    <..190
Exon 8         :    191..306
Intron 8       :    307..>
Sequence       :    412
Remark         :    intron 7 contains CA-repeat (D19S1150)

cccagtcttt tcccagaagt cctgactcct cctgttgaaa actcctgacc      50
tccagggact tctgaatccc caaacacaca cacacacaaa cacacacaca     100
cacacacaca cacacacaca caaacacaca cacaaacgtt tcctaacatt     150
tttaaaacag ccatactctg gcttttctat gcttctccag GGAGTTTGCC     200
AAAGAAAGGG AACGGGTGGA GAACCGGCGG GCTTTTCTGA AGCTGAGGCG     250
GCAACAACAG ATTGAACGTG AGCTCAATGG GTACATGGAA TGGATCTCAA     300
AAGCAGgtga ggccctttca tcctggggcc cagggatgga gatcccaggc     350
cacagagtac aaagagagtc atgcagtttg gagaaggcta agctgggagg     400
gttatgatgg ga                                              412
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Submission no   :     9
Intron 8        :     <..513
Exon 9          :     514..570
Intron 9        :     571..>

gagtaggaag ttagaggcag ggtggtcagg gaaggcttct ctaaggaagt    50
accctctgag cagagagacc tgaaggacgt gaagaaggaa gctgtgggga   100
tgtcaaggga aggggcattc caggcagaga cagcaagtgc aaaggccctg   150
agctaggaac gtatttgaga cacagcaagg aagccagtgc agctgaaaca   200
gagtgagagg tggggacagc tggaggagag gaagacagga aggtgatgga   250
gatcagatca agcaggggct tataggctgt ggtgtggaca ttggtttta    300
ttttgcgcga ggtggggaga atgttggcta ttgctactgt tgcggaggtg   350
gggcttgaag tcacaaacca cccagcagca tgttttttgg tcggttgagc   400
tgtcaccatc agtcagcaga gaatgggggt ggccgggcag acccttcttc   450
ctggtccaag ggagaactca tcctccaaat gcaggagctt aactctgtgc   500
tcttcctctt cagAAGAGGT GATCCTCGCC GAGGATGAAA CTGACGGGGA   550
GCAGAGGCAT CCCTTTGATG gtaactgctc taaacccacc tcaggggtgg   600
gtcccagggg a                                             611
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//
Submission no   :     10
Intron 9        :     <..86
Exon 10         :     87..179
Intron 10       :     179..>

ttaatccaag acacactgtg tgtcctatat ggtctgtgtt cgaaaaaggg      50
taacgtcttt ttctcttgcc atgtttccat tgttagGAGC TCTGCGGAGA     100
ACCACCATAA AGAAAAGCAA GACAGATTTG CTCAACCCCG AAGAGGCTGA     150
GGATCAGCTG GCTGATATAG CCTCTGTGGg tgagtccctt cctctgccac     200
ctatcagttg ttcatcacct atcgcccaag agacatggtg gggtgggggc     250
agagggcttg caaaccgtgc tgcctggatt tgggtctcag ctccaccctt     300
tcccacctgt gcgtgtgtcc tgggcagatt acatcattat gggaataaca     350
tccgtgccta gcttctcatt attttgtggg aattcaacta aatgatcccc     400
atgaagcatg gcaaaccagc acctggcagg gacgaagctc ccagtcaagt     450
tggtgaatgt ttgtgactca ttcgggaagt atcatggggg acctgcttat     500
attaggtgct tggttgcaaa caaacaaggc agtcacgagg ctgagctggg     550
aggatcactt gagcctggga agtggaggct gcaataagcc attattgtgt     600
tactgcactc cagcctggca cagaaaaaaa aaaaaaanac aaactgagcc     650
agcaca                                                     656
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Submission no   :    11
Intron 10       :    <..450
Exon 11         :    451..660
Intron 11       :    661..>

gatcacttct aaagttaaat gtccatggga aaacagtctc atccacatct      50
ctttctggag gccttccaag cgtgctccat gcagctctgt tgcctgcccc     100
tgcatcaggg aatggaggct ctgctttatc ctgccctgtg gtgtgactcc     150
cagaggcatc agatgtggct gggagtggga gacatggaaa attggctcct     200
gcaacagaga actatcagcc ttcccatcaa ttggttactt ctaattctgt     250
tatttttcag gggcactgtc ttctcataag ctccatctat gcaaaactaa     300
gcccatgggt catgatggtt ccctcaggcc agaggcttgc tggagagact     350
aatggatccc ctggctaaaa tctgtgcttg ggctgcacat tggttaattt     400
cttctgaagg aacagcctga gcctgacatt ctccatcttt tccctggcag     450
GTTCTCCCTT CGCCCGAGCC AGCATTAAAA GTGCCAAGCT GGAGAACTCG     500
ACCTTTTTTC ACAAAAAGGA GAGGAGGATG CGTTTCTACA TCCGCCGCAT     550
GGTCAAAACT CAGGCCTTCT ACTGGACTGT ACTCAGTTTG GTAGCTCTCA     600
ACACGCTGTG TGTTGCTATT GTTCACTACA ACCAGCCCGA GTGGCTCTCC     650
GACTTCCTTT gtgagtatca cccagcccca cccctgccaa ctccctgatc     700
cctccctcac acccttttc cacttctctt tctctggtag tatgtgtatc      750
ttctttggtc ctcattgaat ctgcccct                             778
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Submission no   :    12
Intron 11       :    <..323
Exon 12         :    324..436
Intron 12       :    437..>

gatcacttgt ggccaggagt tcaagancag ccagggcaac atagtgagga     50
cccccatctc cacattaaaa attttaaaaa gaaaaaagat aagtcagaag    100
ttgggtgtgg tgacacatgc ctgtagttct agcatgttgg aggccaaatc    150
agggaaactg tttgaggcca ggagtttgaa accagcctaa cagcatagca    200
agacctcatc tctacaaaaa ataaaaagtt taaaaatgat aataaaagga    250
aagtcagagc cacctggaac ccctaccctc agcaagccta acctcctctc    300
tgtttcctcc ttctcccttc tagACTATGC AGAATTCATT TTCTTAGGAC    350
TCTTTATGTC CGAAATGTTT ATAAAAATGT ACGGGCTTGG GACGCGGCCT    400
TACTTCCACT CTTCCTTCAA CTGCTTTGAC TGTGGGgtaa gtgctcttgt    450
ttctaagagt tcatttctcc agctcttgcc tggaatgaca gatacctgga    500
cacattaaag ggagaaaggt aaagtcaccc ctgaatatga gagactcaga    550
tggatgcaga aggaatgaga aaacaatcca aacactggca aggatacagt    600
gtacccagaa ccctcaacca ccgcca                              626
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Submission no   :   13
Intron 12       :   <..545
Exon 13         :   546..658
Intron 13       :   659..821
Exon 14         :   822..953
Intron 14       :   954..>
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gatcngncat gcacaccagc ctgggtgata agagcaagac tcctctcaaa    50
ataaatgaat aaataaaaat aaataaataa ataagaggcc gggtgcagtg   100
gctcaatgct ttggaaagtg gaggccaaca gttggagaga ccaaagcagg   150
aggatggctt cagcccagaa gtttgaggcc mgcctgggca atactagcga   200
gacactatct ctataaaaat gttttaaaat tagccagatg tggtggggca   250
cacctgtaat cccagctact caagaggctg aggtgggagg atcacttaag   300
cccaggagga cagtgctgca gtgagctatg attgcgccca ctgcactcca   350
gcctgggtga cacagtgaga cccggtctct atagataaat gaatggatga   400
atgagggggt caaggatcct cacccggctt ccatttggag ggaggagttt   450
ggttgagttc ttgcaaggtt ggtacctagg aaatgcttgc cagttctgga   500
gcccagacac tgtccctgga catgagacca ggttctctgc cctagGTTAT   550
CATTGGGAGC ATCTTCGAGG TCATCTGGGC TGTCATAAAA CCTGGCACAT   600
CCTTTGGAAT CAGCGTGTTA CGAGCCCTCA GGTTATTGCG TATTTTCAAA   650
GTCACAAAgt aagtctttgg ggttcctgga catttgtaca gggggtgggg   700
atgggggaca tggtggggcc gcctccagaa agttgggaaa gtgagcctcg   750
tgtttcgagg gctgactccg gggcctgcct wccccgcctg gcctgagtcc   800
tcgcctggsc tctgtcggca gGTACTGGGC ATCTCTCAGA AACCTGGTCG   850
TCTCTCTCCT CAACTCCATG AAGTCCATCA TCAGCCTGTT GTTTCTCCTT   900
TTCCTGTTCA TTGTCGTCTT CGCCCTTTTG GGAATGCAAC TCTTCGGCGG   950
CCAgtaagtc cttcacagga attcaa                             976
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Submission no   :    14
Intron 14       :    <..201
Exon 15         :    202..274
Intron 15       :    274..>

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agatgatgga aacgttcgtt tccttggaga tgagatactg agccacaccc     150
tcagagcacc ccgggtgggg ccaacgtgaa atgtctgtgt cctccctgca     200
gGTTTAATTT CGATGAAGGG ACTCCTCCCA CCAACTTCGA TACTTTTCCA     250
GCAGCAATAA TGACGGTGTT TCAGgtacag cctccacctg gccccacggg     300
ccaacacctc tcagtgtcac agatgaaagt gcctgctcca catccaaggg     350
gcttccctga actcctcctt ctctacctgg ccttttcaca ccactttgaa     400
acacagattt tatggttatc attattcaat tatggtgagg ccaacagatc     450
aggagatgaa tgtcattgga aagatagttt gtggctgggc acggtggctc     500
acacccataa tcccagcact ttggccaggt acggtggctc acacctgtaa     550
tcccaacgct ttgggaagcc caggtgggcgg atcacttga gatcaggaat     600
tcgagaccag cctggccaan atggtgaaac cccatctcta ctaaaaatac     650
aaaaattagc cgggcgtggt agcacatgcc tgtaatccca gctactcggg     700
agatgaggca caagaattgc ttgaacctgg gaggcagagg ttgcagtgag     750
ccaagatcgc gccactgcac tcmagcctgg gcaacagagt gagactccat     800
ctcaaaaaag caaaagaaaa aaaaaaccac tttgggaggt caagatggga     850
ggactacttg aggccaggag tttgagacaa gtctgggcaa catagtgaga     900
ctccgtctct gcaaaaaaat wataataata attagctggg catggtgata     950
catacctcct agctactagg gcagctgaag tggaaggatt gcttaagccc    1000
aggaggttga ggctgcagta agctacaatc acaccactat actccagcct    1050
gggcgagaga gcaaagccct gtctcaaaaa cgaaaagaaa gtttgttata    1100
ctcacagatc                                                1110
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Submission no   :      15
Intron 15       :      <..524
Exon 16         :      525..642
Intron 16       :      643..795
Exon 17         :      796..863
Intron 17       :      864.>

gatcctccca ccttggcctc ccaaagtgct gggattacag gcatgagcca      50
tggcatgcgg tctcttcctg ttcttataag ggcactaata ccatcatgaa     100
gtccccccatg acctcatcta accctagtta cctcttaaag gccccatctc    150
caaataccat cccatcatag gttagggctt caactcatga atttggaggc     200
gggcacaatt tagtccataa caaatcccct taatcacatc aagtaagaca     250
gagttacagg agggtctgtg actcctccag ggtcccattt tcctagaagc     300
caggctaaga gccccacgac gcaggaacgg ccctttctac tcgcaaacaa     350
agagaaaagc caaggagaag ccaacacgga gtctggctct gcaaaccggg     400
caggattgtt aaagacctcc tgggctcggg gatggggtgg gcggattccg     450
gctccacagc tgcatctcca aggggcccgt ggctgagagg ggggttggct     500
gtgtgtttct tcctcccctt tcagATCCTG ACGGGCGAAG ACTGGAACGA     550
GGTCATGTAC GACGGGATCA AGTCTCAGGG GGGCGTGCAG GGCGGCATGG     600
TGTTCTCCAT CTATTTCATT GTACTGACGC TCTTTGGGAA CTgtatcctt     650
catggagaga gagaagggga caggcctgga cctctggcag aggagaggtt     700
gcaggggctc aagggagggt actgagagac ccagataccc aggcccaag      750
tggtgtccca ccagtggttg cttttcctga ctcagacatt tgcagACACC     800
CTCCTGAATG TGTTCTTGGC CATCGCTGTG GACAATCTGG CCAACGCCCA     850
GGAGCTCACC AAGgtggagg cggtgggaga atgtttctct ggcaaagtta     900
ccacctgccc atggcagatc aagcactttt ttggattaac tgagccacag     950
gaaataacat tttcaaatag atkaaaaaga tc                        982
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//
Submission no   :    16
Intron 17       :    <..119
Exon 18         :    120..226
Intron 18       :    227..>

     ccttggttct gattggtcga aatatttcaa atgttgcccc tggtcagcaa      50
     cagggtcaga agtgagtccc caaggcctag ttcatgtttt gtgaacaaag     100
     attccacgtg ccttttcagG ACGAGCAAGA GGAAGAAGAA GCAGCGAACC     150
     AGAAACTTGC CCTACAGAAA GCCAAGGAGG TGGCAGAAGT GAGTCCTCTG     200
     TCCGCGGCCA ACATGTCTAT AGCTGTgtaa gtcccctaat ccctgggatg     250
     ctaccctggc tcctgaacgt gtccgaccac tatccaggca cagatttggg     300
     aagcagtggg ggtg                                            314
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Submission no : 17
Intron 18 : <..209
Exon 19 : 210..1019
Intron 19 : 1020..>

gcccctagcc aggtgggagc catggagggt tcttgagcag aggaggctgg 50
gacctgactc agatgctcac agactcctag cattcaggtg gggagtagag 100
ggtggagagc aggagtggga ggctgagatg tgggttggtt cgcctgggtc 150
atccatccaa gctacagtgc ctagcaatgc tctaagctcc tgtgaccatg 200
ccactgcagG AAAGAGCAAC AGAAGAATCA AAAGCCAGCC AAGTCCGTGT 250
GGGAGCAGCG GACCAGTGAG ATGCGAAAGC AGAACTTGCT GGCCAGCCGG 300
GAGGCCCTGT ATAACGAAAT GGACCCGGAC GAGCGCTGGA AGGCTGCCTA 350
CACGCGGCAC CTGCGGCCAG ACATGAAGAC GCACTTGGAC CGGCCGCTGG 400
TGGTGGACCC GCAGGAGAAC CGCAACAACA ACACCAACAA GAGCCGGGCG 450
GCCGAGCCCA CCGTGGACCA GCGCCTCGGC CAGCAGCGCG CCGAGGACTT 500
CCTCAGGAAA CAGGCCCGCT ACCACGATCG GGCCCGGGAC CCCAGCGGCT 550
CGGCGGGCCT GGACGCACGG AGGCCCTGGG CGGGAAGCCA GGAGGCCGAG 600
CTGAGCCGGG AGGACCCCTA CGGCCGCGAG TCGGACCACC ACGCCCGGGA 650
GGGCAGCCTG GAGCAACCCG GGTTCTGGGA GGGCGAGGCC GAGCGAGGCA 700
AGGCCGGGGA CCCCCACCGG AGGCACGTGC ACCGGCAGGG GGGCAGCAGG 750
GAGAGCCGCA GCGGGTCCCC GCGCACGGGC GCGGACGGGG AGCATCGACG 800
TCATCGCGCG CACCGCAGGC CCGGGGAGGA GGGTCCGGAG GACAAGGCGG 850
AGCGGAGGGC GCGGCACCGC GAGGGCAGCC GGCCGGCCCG GGGCGGCGAG 900
GGCGAGGGCG AGGGTCCCGA CGGGGGCGAG CGCAGGAGAA GGCACCGGCA 950
TGGCGCTCCA GCCACGTACG AGGGGGACGC GCGGAGGGAG GACAAGGAGC 1000
GGAGGCATCG GAGGAGGAAg taagtggagg tgacctcgaa tccgcagaat 1050
gacggtaaca ttaataatac aacagccaaa gtagcacgtg ctgtgtattt 1100
gttataaaat ata 1113

```
//
Submission no   :   18
Intron 19       :   <..67
Exon 20         :   68..531
Intron 20       :   532..>

gtcctgaaac tttgcctttt aatcctaaat cattgttggt tctttttcat    50
tcacttgcct tcctcagAGA GAACCAGGGC TCCGGGGTCC CTGTGTCGGG   100
CCCCAACCTG TCAACCACCC GGCCAATCCA GCAGGACCTG GGCCGCCAAG   150
ACCCACCCCT GGCAGAGGAT ATTGACAACA TGAAGAACAA CAAGCTGGCC   200
ACCGCGGAGT CGGCCGCTCC CCACGGCAGC CTTGGCCACG CCGGCCTGCC   250
CCAGAGCCCA GCCAAGATGG GAAACAGCAC CGACCCCGGC CCCATGCTGG   300
CCATCCCTGC CATGGCCACC AACCCCCAGA ACGCCGCCAG CCGCCGGACG   350
CCCAACAACC CGGGGAACCC ATCCAATCCC GGCCCCCCCA AGACCCCCGA   400
GAATAGCCTT ATCGTCACCA ACCCCAGCGG CACCCAGACC AATTCAGCTA   450
AGACTGCCAG GAAACCCGAC CACACCACAG TGGACATCCC CCCAGCCTGC   500
CCACCCCCCC TCAACCACAC CGTCGTACAA Ggtgagaccc tctgctctca   550
catcactggg caggggacct ggcgtcctgg agccagaggt              590
```

```
//
Submission no   :     19
Intron 20       :     <..75
Exon 21         :     76..217
Intron 21       :     218..>

ggagtacacc gaggagttcc cagagacttg tgggaaattg tggagggagc     50
cctgtgttgg ttcttgtccc aacagTGAAC AAAAACGCCA ACCCAGACCC    100
ACTGCCAAAA AAAGAGGAAG AGAAGAAGGA GGAGGAGGAA GAAGACGACC    150
GTGGGGAAGA CGGCCCTAAG CCAATGCCTC CCTATAGCTC CATGTTCATC    200
CTGTCCACGA CCAACCCgtg agtatggccc ccgagcagag ggcagggggg    250
gctgggtctc ccaccagggt ggcggaannn nnnnnnnnnn nnnnnnnctc    300
ccaccagggt ggcggaagtc aggccagatt agagggcaat               340
```

```
//
Submission no    :     20
Intron 21        :     <..97
Exon 22          :     98..227
Intron 22        :     228..>

gatctcagta gtggtaggta acatgagatt atggaagaaa agggtttgtg     50
agcctgtggt ctgagtggac ctctgcacgc ccatctgtct ccaacagCCT    100
TCGCCGCCTG TGCCATTACA TCCTGAACCT GCGCTACTTT GAGATGTGCA    150
TCCTCATGGT CATTGCCATG AGCAGCATCG CCCTGGCCGC CGAGGACCCT    200
GTGCAGCCCA ACGCACCTCG GAACAACgtg agtcccacag agcacacccc    250
ttcctagcct ggctgctctg cctcaggcca ctttctcctg catccaaaat    300
gctcataggt agggtgggat gttggggtca cccctaggca tagcccttat    350
ggctgctggt tgagagggga agctctgatt ccttggggat gctcttggga    400
gcaagacatt ccttgaggca gtttctctgt gagcctggtg gggtggaggt    450
ggcccagagt gactggggct gaaaatt                             477
```

```
//
Submission no  :   21
Intron 22      :   <..33
Exon 23        :   34..93
Intron 23      :   94..>

     gatccactgc tctcttgctt ttatccctta cagGTGCTGC GATACTTTGA        50
     CTACGTTTTT ACAGGCGTCT TTACCTTTGA GATGGTGATC AAGgtgagtg       100
     cagattataa gtgagaacac acggtaattt tttttttaa gcaagtgcag        150
     ggctgggcac agtggatc                                          168
```

```
//
Submission no  :    22
Intron 23      :    <..232
Exon 24        :    233..339
Intron 24      :    340..>

gatctaagag ccggcaagcc agagctggct tccatcaggc aaagggggc     50
cgcctcatgg ggcaggggct ccccactcct ccctgggagt cctctggcca    100
ctgcccatcc ctgcaagatg aggtggcctc attggcttcc ctgcctctcc    150
ccgagaggct agagagtggg tggcagcacc ccagggtggg gatcaggtgg    200
gggttctgag caccctctct tctcccccac agATGATTGA CCTGGGGCTC    250
GTCCTGCATC AGGGTGCCTA CTTCCGTGAC CTCTGGAATA TTCTCGACTT    300
CATAGTGGTC AGTGGGGCCC TGGTAGCCTT TGCCTTCACg taagtctctt    350
cgcaagggtt tcctcttg                                       368
```

```
//
Submission no   :     23
Intron 25       :     <..244
Exon 25         :     245..344
Intron 24       :     345..>

gatcttaacc ccaagacact tcatctaaag gaaaaactgc cataatacac     50
agattatttt aggtcagctc actttactgc catctgctgg gaagttgtaa    100
taatacaaat atccatacac gatggctagg atgttatcag cacctccttt    150
aatgtgttgt ccttgagcag tgtacaacct gctcagctgt acatgataac    200
cctgacagtc ccccccaccg caccccacca tctcccaatc tcacCTTGAG    250
CTTTGGCAGC CGCTTGATGG TTTTAAGAGG TCGTAGCACC CGGAGGACTC    300
GGAGGGATTT AATCGTGTTG ATGTCTTTTC CTTTGCTATT GCCActgtgg    350
aggaatgttt aggtgggaag aagggaagag aggaagcaga ggtcaggttg    400
ggtagggggc agcccacagc tccatgggac cctacccttc ccaggcctag    450
aagtctgggg tgagcttggc acaagcctgc cctttcctgg tgaagagtgg    500
tccattttac cctgt                                          515
```

```
//
Submission no   :   24
Intron 25       :   <..67
Exon 26         :   68..228
Intron 26       :   229..>

ggccactgga ggcagaaggt tggcaggtcc ccagcccctc atgctctctg    50
tcaactccac cccacagGCT GTGTTTGACT GTGTGGTGAA CTCACTTAAA   100
AACGTCTTCA ACATCCTCAT CGTCTACATG CTATTCATGT TCATCTTCGC   150
CGTGGTGGCT GTGCAGCTCT TCAAGGGGAA ATTCTTCCAC TGCACTGACG   200
AGTCCAAAGA GTTTGAGAAA GATTGTCGgt gggtctccgc tttccagcac   250
attcccattg gaaccagcag gtgggcaggg gggaagtggc tagaggcatt   300
ggccacttgg gctcagagac tggagaagtg atgagccttg gaagtgactc   350
agttgcaacc agcttggatc aagggtagaa agaaaaccgg ttttagaatt   400
tgagtc                                                   406
```

```
//
Submission no  :    25
Intron 27      :    <..177
Exon 27        :    178..315
Intron 26      :    316..>
Remark         :    reversed direction!

     gatctcaaac tcctggcctc aagtgataca tctgccttgg cctcctaaag      50
     tgttgggatt acaggcgtga gcaccatgcc cggcctccaa gacctttctt     100
     attgctaagc tctcaggccc tttatcctcc tgctccccag ggctcctcct     150
     ggatagattt ccagtcgggc cacttacTGT GGCCAGCCTT CTCCCGTGGA     200
     CACGGTGAAG AGGGTCAGCA GAGCCCACAG CACATTGTCG TAATGGAATT     250
     CATACTTCTT CCACTCCCGG TCTCGCGCCT TCACCTCATT CTTCTCGTAG     300
     AGGAGGTATT TGCCTctgcc acagagagtg gggactgtta gtaaatggga     350
     aagaggggct gtcttgcact tgtctttggt tatcagagac agggggaggg     400
     aaaggaagag tggtccacca ncctagactg cttgggaagc agtgacttcc     450
     catcctgcca ccatgtgttc ctgtgcttca taggggatgn cgtgtgcaat     500
     ctacttttna ggataa                                          516
```

```
//
Submission no   :    26
Intron 27       :    <..84
Exon 28         :    85..276
Intron 28       :    277..>

acttcctca tcacccttgg gtccctgtct ctctccttcc tgccccttcc     50
ctctccctgc cccattcctt gcagGGTCCT CAAGCATTCG GTGGACGCCA   100
CCTTTGAGAA CCAGGGCCCC AGCCCCGGGT ACCGCATGGA GATGTCCATT   150
TTCTACGTCG TCTACTTTGT GGTGTTCCCC TTCTTCTTTG TCAATATCTT   200
TGTGGCCTTG ATCATCATCA CCTTCCAGGA GCAAGGGGAC AAGATGATGG   250
AGGAATACAG CCTGGAGAAA AATGAGgtgc cacttccaat tccatctgtc   300
ctttaaaaac tggggacaca cacaaacttt aaaacacaca caacacccag   350
gaacccctttt ctaggggtac ctgggggagg gaacagaagc attgtcccaa   400
ccgaatccag tcttcagggc agcccttcat ggagtttcag aggaaacaca   450
tcatatagtg tatgtatcag tcagtttaga ctaggttat                489
```

```
//
Submission no   :     27
Intron 28       :     <..253
Exon 29         :     254..418
Intron  29      :     419..>

tagcccatgc aanaatgggg aaatgncagt gcaagttttg gcagttgntg         50
acatctcaag caactgtanc tgttgggata agaaagcaat ggtgagaagg        100
aanagaganc ccaggaatcc tggctggggg caananaggc agagactcaa        150
gcagaagcac ttgagaaccg cgacgagtta gacagagggt gcccggtgta        200
cagccacctt cctcctgcct ctgccgctct caccactggc ctctctcccg        250 50
cagAGGGCCT GCATTGATTT CGCCATCAGT GCCAAGCCGC TGACCCGACA        300
CATGCCGCAG AACAAGCAGA GCTTCCAGTA CCGCATGTGG CAGTTCGTGG        350
TGTCTCCGCC TTTCGAGTAC ACGATCATGG CCATGATCGC CCTCAACACC        400
ATCGTGCTTA TGATGAAGgt aagtgcccca caccagcccc cagcactant        450
taacccccac ctcgttcctg cctctaccct gataaaatga aaccatttgc        500
agatttccca ga                                                 512
```

//
Submission no : 28
Intron 29 : 156
Exon 30 : 157..267
Intron 30 : 268..>

```
gggtctttcc tgaactgtgc ctcctaccag tgaggttgct cagaccttgc     50
ctggggctgg agtgttgcct ggagaacagc catgaagctg acctccccac    100
ttcccacttc ccacccctgc tcgctgaccc ctgctactcc tgcttctttc    150
ccctagTTCT ATGGGGCTTC TGTGGCTTAT GAAAATGCCC TGCGGGTGTT    200
CAACATCGCC TTCACCTCCC TCTTCTCTCT GGAATGTGTG CTGAAAGCCA    250
TGGCTTTTGG GATTCTGgta agtaccacct tggggctaca gctatgggct    300
tggnanaanc ccaaggggga acaatgggtc ctggatgatg gtctcccaac    350
gtggccccaa gaaccccaac ctcaagggtg gcttcagtat cctgcccagt    400
ggccacagat c                                              411
```

//
Submission no : 29
Intron 30 : <..115
Exon 31 : 116..199
Intron 31 : 200..>

```
ctgtcccggg cactccgctg atgggcaact gtgcctctaa catgcaccgg    50
ccagcctagg gggccgggaa ccaagccctc tgttggcatc tctgtcttgt   100
gggtccccat tctagAATTA TTTCCGCGAT GCCTGGAACA TCTTCGACTT   150
TGTGACTGTT CTGGGCAGCA TCACCGATAT CCTCGTGACT GAGTTTGGGg   200
taagtctccc tccagcttct ctctgggtga ctctgggctg gacgaggcag   250
gcggcagggg gcgggggagc ggtcccagag gcagtgtgtc ccggaagcca   300
tagctgcttg agccagcact tggccatgac cagagaggga gaactggggc   350
cccggggaca agggcagccc ctcaggaggg cattgtgggg agatgggggt   400
aacaaagctt ggctgtaggg                                    420
```

```
//
Submission no  :    30
Intron 31      :    <..148
Exon 32        :    149..265
Intron 32      :    266..>

ttaatagtgc tttctctctc cctccttatt tggggtctgg cttgcttttt    50
tcctgttggt tggcttcatg taggggcctc tgtgagtggt gacagctctg   100
agcctttggg gtgggtggat ggtcacccct cttcctccat ctccccagAA   150
TAACTTCATC AACCTGAGCT TTCTCCGCCT CTTCCGAGCT GCCCGGCTCA   200
TCAAACTTCT CCGTCAGGGT TACACCATCC GCATTCTTCT CTGGACCTTT   250
GTGCAGTCCT TCAAGgtgag tcctcgtccc tgctgctggc ccaggggctg   300
agaagacagg tgaccctcat gctctggctg aatgtagaag tc           342
```

```
//
Submission no   :    31
Intron 32       :    <..156
Exon 33         :    157..222
Intron 33       :    223..394
Exon 34         :    395..509
Intron 34       :    510..>

cccccaagaa gaatgcccac caagccctgg aaggactctg gcacgtggca      50
tatggccacc caacccagtg gggcagagca ctgggacaag ggaggaagac     100
tgcagtgcgg ctgagggacc cccagcactc ttcttcattg cctttttcc      150
caccagGCCC TGCCTTATGT CTGTCTGCTG ATCGCCATGC TCTTCTTCAT     200
CTATGCCATC ATTGGGATGC AGgtgagtgt cgtgtcccta aggttcccag     250
agcctcccaa ggagggcagc caccccttaga aaggggtggg tcagaggagc    300
ctggttcaca gaagcagcca tggaggttga gctgggtttc ccagaagcca     350
ctggaggaat ggcagcccct ggtcgtcacc cwmcaattcc acagGTGTTT     400
GGTAACATTG GCATCGACGT GGAGGACGAG GACAGTGATG AAGATGAGTT     450
CCAAATCACT GAGCACAATA ACTTCCGGAC CTTCTTCCAG GCCCTCATGC     500
TCTCTTCCGg tcagaagggg acctgctctg ataatnctgt ttccgtgggg     550
tggggtgcc                                                  559
```

//

```
Submission no  :    32
Intron 34      :    <..94
Exon 35        :    95..245
Intron 35      :    246..>
Sequence       :    316

tcagagccat gctcactgtg tgctccactc ctgaggaggc gttggtacca     50
gtcagggctg ggtgtccgag tctctgattt ctccctgtcc tcagGAGTGC    100
CACCGGGGAA GCTTGGCACA ACATCATGCT TTCCTGCCTC AGCGGGAAAC    150
CGTGTGATAA GAACTCTGGC ATCCTGACTC GAGAGTGTGG CAATGAATTT    200
GCTTATTTTT ACTTTGTTTC CTTCATCTTC CTCTGCTCGT TTCTGgtgag    250
tctgtggaca ctgtgagggc cgtctgggct ccctaagcct ggcttccttt    300
cagggagtgg ttctgt                                         316
```

```
//
Submission no  :    33
Intron 35      :    <..211
Exon 36        :    212..339
Intron 36      :    340..>

gtgtagtgag aactcacctc tccattcccc agtctctttc tgtctctgtc     50
tcatttcctt tccccatctt ctctctatcc ctctctccat ctggggcctc    100
tgtgtctgtc tttgggtctg tctgtccgtc tgactgtctg tatccttctc    150
acttcactca ttcattccct cggtctctgc cccattctct cttggtcccg    200
ggtccccaca gATGCTGAAT CTCTTTGTCG CCGTCATCAT GGACAACTTT    250
GAGTACCTCA CCCGAGACTC CTCCATCCTG G3CCCCCACC ACCTGGATGA    300
GTACGTGCGT GTCTGGGCCG AGTATGACCC CGCAGCTTGg taagaagtca    350
ccccgaatcc tccagccaca atactcacct ctccctggaa ctggaacacg    400
ggctaggcta ggnccccaga ctctggagca ctgaactcct ggggctccta    450
gcaggggtct cacaggttca gtcaggagag aagatataag aatcatcacc    500
cttgcatacc ccagattaaa cacgtagggt gccaacctgc ccaaaccctg    550
gaggactttc tgggaaatga ggagggcgtc aaccatgaga tgtctgaaga    600
gccctctcct cctacgagtc tctcctgtct ctcactgtga agtctccaga    650
tggtgaggat cgattagcca ggctccagga gaaaccaaca gact          694
```

```
//
Submission no   :     34
Intron 36       :     <..213
Exon 37         :     214..310
Intron 37       :     311..>

aagggaggtg cctgcagtcc cgaactcgac tgacatccta cacccctggg     50
tctccccagt gtctgggaat gtactgggaa ttcacttgtc cccagtctct    100
cccactcctt gaagccaggg acaccccagc ctcgggcatc atgacctcgt    150
tgtgtgccca gggagcccgt gtgaacccat tgcctgcact aaccccettt    200
cttctccttt cagCGGTCGG ATTCATTATA AGGATATGTA CAGTTTATTA    250
CGAGTAATAT CTCCCCCTCT CGGCTTAGGC AAGAAATGTC CTCATAGGGT    300
TGCTTGCAAG gtttgacttc cactaaaacc tgctagcatc catggaatga    350
gtgtggcttg gggttcttca atatatatat ttcatatata tatatatata    400
tatctctctc tctctaaaaa aacagagcca tctctctttc ttgcattaaa    450
ctagaaaact ctcttagcca acag                                474
```

//
Submission no : 35
Intron 37 : <..82
Exon 38 : 83..188
Intron 38 : 189..>

```
cctgggtagg ggcgggcgcg gctcacggga gacccaggag ggatgcctgg   50
gaatgactgc gcttgccttg ggttttctgt agCGGCTTCT GCGGATGGAC  100
CTGCCCGTCG CAGATGACAA CACCGTCCAC TTCAATTCCA CCCTCATGGC  150
TCTGATCCGC ACAGCCCTGG ACATCAAGAT TGCCAAGGgt aaggaaggga  200
caggggcggg cacagacagg cgtgacaggg tggaactggg gatctcctcc  250
ctaccccaaa ctagaggatc tgctgtcacc acccggatct tcattcactc  300
ttccattcat tcgttccaca ggnntttttg gnnnttggnn ntttggtgtt  350
tttttttttt ttttgagaca gagtcttgct ctgttgccca ggcagcagtg  400
cggtgacatg atc                                          413
```

```
//
Submission no   :     36
Intron 38       :     <..96
Exon 39         :     97..204
Intron 39       :     205..369
Exon 40         :     370..470
Intron 40       :     471..>

gggtctcgtt ctcgggagcc tatggctttg cagctgaccc agagtccagc     50
tgacacccag gcaggcagtc agggtctgtc tacaccccca ttgcagGAGG    100
AGCCGACAAA CAGCAGATGG ACGCTGAGCT GCGGAAGGAG ATGATGGCGA    150
TTTGGCCCAA TCTGTCCCAG AAGACGCTAG ACCTGCTGGT CACACCTCAC    200
AAGTgtaaga gctgagccca gccctgggat ccaatccacc aggacagatg    250
gag[illegible]gggagg gaaaggggag gcctggggag agtgttggct gggctggtat    300
acacagggac ccaggacaag gtccccaaag angcctgccc ttggtgagct    350
caccgtgtgt gtcccccagC CACGGACCTC ACCGTGGGGA AGATCTACGC    400
AGCCATGATG ATCATGGAGT ACTACCGGCA GAGCAAGGCC AAGAAGCTGC    450
AGGCCATGCG CGAGGAGCAG gtgcgctgtt cgccgctctg gggacatctg    500
ggctggggac agtggcttgc atgtcaccac gggaaccaac tggaatatga    550
gggtggctga gccccagggc aggtccctga aaagtagggg ctggtgcaca    600
gcagctcaca cctgcaatct cagtgctttg agaggc                   636
```

```
//
Submission no   :    37
Intron 40       :    <..407
Exon 41         :    408..517
Intron 41       :    518..625
Exon 42         :    626..764
Intron 42       :    765..>
Sequence        :    829

     gatcttcagg gccatgggag ctgcaggaag gactctggct ttttccccaa        50
     gcaagtggga gccatggagg gttctaagca aaggagggat aggacctgac       100
     tcaagtgctc atgggcgccc tctggtggct cttgtggaac agtggggttg       150
     aaggtaggag cgggagacct gggagaaggt gcctgcagtg agagatgagg       200
     acgcgggacc aggctggggc tatgacttgg gtggaggagt gagaagtggt       250
     ccagttctgc gtggaattgg aagggtctag atggatgaga cctgagagag       300
     tgtgtgtgtg tgtgtgtgtg tatactgggg atgtcgcaat gccttctggg       350
     taccaccgtc caccacccca cccttgtcca cacactgctc tctgccccat       400
     tccccagGAC CGGACACCCC TCATGTTCCA GCGCATGGAG CCCCCGTCCC       450
     CAACGCAGGA AGGGGGACCT GGCCAGAACG CCCTCCCCTC CACCCAGCTG       500
     GACCCAGGAG GAGCCCTgtg agtgtcaccc ctgccaggga ggtggagtgt       550
     gggggtgccg tggtccccac gttctggaag ctgcccaagc gcccactgct       600
     accccggcct ctgtcccccca tgcagGATGG CTCACGAAAG CGGCCTCAAG      650
     GAGAGCCCGT CCTGGGTGAC CCAGCGTGCC CAGGAGATGT TCCAGAAGAC       700
     GGGCACATGG AGTCCGGAAC AAGGCCCCCC TACCGACATG CCCAACAGCC       750
     AGCCTAACTC TCAGgtgcct ctgtccccca actccccaat ggctcccagg       800
     gcccgggtgg ttgcggtgga aggaaccat                              829
```

```
//
Submission no  :    38
Intron 42      :    <..219
Exon 43        :    220..333
Intron 43      :    334..>

tcactgcaac ctccaccttc cagtctcaag tgattcctcc tgcctcagcc      50
tcccaagtca ctggattaca ggcgcccacc accatgctca ggtatttttt     100
tttgtatttt tagtagagac ggggtttcac aatgttggtc aggctggtct     150
cgaactgctg nccattgtga tctggaggtc aggccccaga gctcatctgg     200
ctttgccatt cgtccgcagT CCGTGGAGAT GCGAGAGATG GGCAGAGATG     250
GCTACTCCGA CAGCGAGCAC TACCTCCCCA TGGAAGGCCA GGGCCGGGCT     300
GCCTCCATGC CCCGCCTCCC TGCAGAGAAC CAGgtgaggg ctttcaccac     350
tgccctgggg ctggacccct cactctgcac tgggtagggc caggcccccc     400
cacaagcagc ccagtgcatc ccctcctgcc ggactcaggc ctgggtaggg     450
actccttcag tctctgaagc agtctgcagg ccccacccac cacctggtca     500
cacctggagc acctgcagac cctcctccct cacagaggac agagaggaaa     550
gtgctccccc tggggcagag ggcagtggcc actgcaaaat ggtctctggc     600
tgccctggtt ggaggctgca gacaggggag gttgtggaar atttgtgggt     650
gcagcagggt tcaacagggc cagctgagac ctgccacgaa gawcctttga     700
ggccaggagt ttgagaccag gttgggcaac atagcaaaac cctgtctctt     750
tttttttttt gagacggagt ttcactcttg ttgccccagg ctggagtgac     800
a                                                          801
```

```
//
Submission no   :   39
Intron 43       :   <..83
Exon 44         :   84..119
Intron 44       :   120..>
Sequence        :   329

cctcctcact cttccctctt gcctttatat ttattttctt ctttctgttt    50
tttctgtgtg caccatccat ggggctgtga cagAGGAGAA GGGGCCGGCC   100
ACGTGGGAAT AACCTCAGTg tatgtacggc ctgcccaggg cccagcaggc   150
tccggccccc tcttcctccc caccccncct ccagggagtc ccgtaatctc   200
taccggtccc cggaccccac cctttctttg gcaatcgcac cctctcccct   250
ccatggagcc caatccttgt gtgtggtgtc ctgtgtgtgc cctgacccat   300
aagcctggtg gggcggccat ccccatcct                          329
```

```
//
Submission no  :   40
Intron 44      :   <..166
Exon 45        :   167..353
Intron 45      :   354..>

gatcaggggg agccaaggcc ccatggcatc ccctggcccc tgccccagga    50
tggtcacacc gcagtcaccg aaggccacca ccaggctgcc acaatggggc   100
aggaaggacc gggaccactt ggtgctagct gctgacccca gcccaccggc   150
ctgtcccctc ccccagACCA TCTCAGACAC CAGCCCCATG AAGCGTTCAG   200
CCTCCGTGCT GGGCCCCAAG GCCCGACGCC TGGACGATTA CTCGCTGGAG   250
CGGGTCCCGC CCGAGGAGAA CCAGCGGCAC CACCAGCGGC GCCGCGACCG   300
CAGCCACCGC GCCTCTGAGC GCTCCCTGGG CCGCTACACC GATGTGGACA   350
CAGgtgggca gccctgtggt gctcagggac aagcagaaca gaggagagga   400
gaggggagga gaaggcaggg cggaggagac actaaggaag aagaaaggga   450
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gatc                                                     554
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Submission no   :    41
Intron 45       :    <..31
Exon 46         :    32..285
Intron 46       :    286..>

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GGGGCCGGCC CAAGGATCGG AAGCATCGAC AGCACCACCA CCACCACCAC     150
CACCACCACC ATCCCCCGCC CCCCGACAAG GACCGCTATG CCCAGGAACG     200
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accccatcca acagccactc taggcaaagg tccccggatc ccggctgtga     400
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Submission no   :     42
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Exon 47         :     280..>
Stop codon      :     280..282
UTR 3'          :     283..>

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ATCCGTAAGG CCGGCGGCTC GGGGCCCCCG CAGCAGCAGC AGCAGCAGCA     450
GCAGCAGCAG CAGGCGGTGG CCAGGCCGGC CGGGCGGCCA CCAGCGGCCC     500
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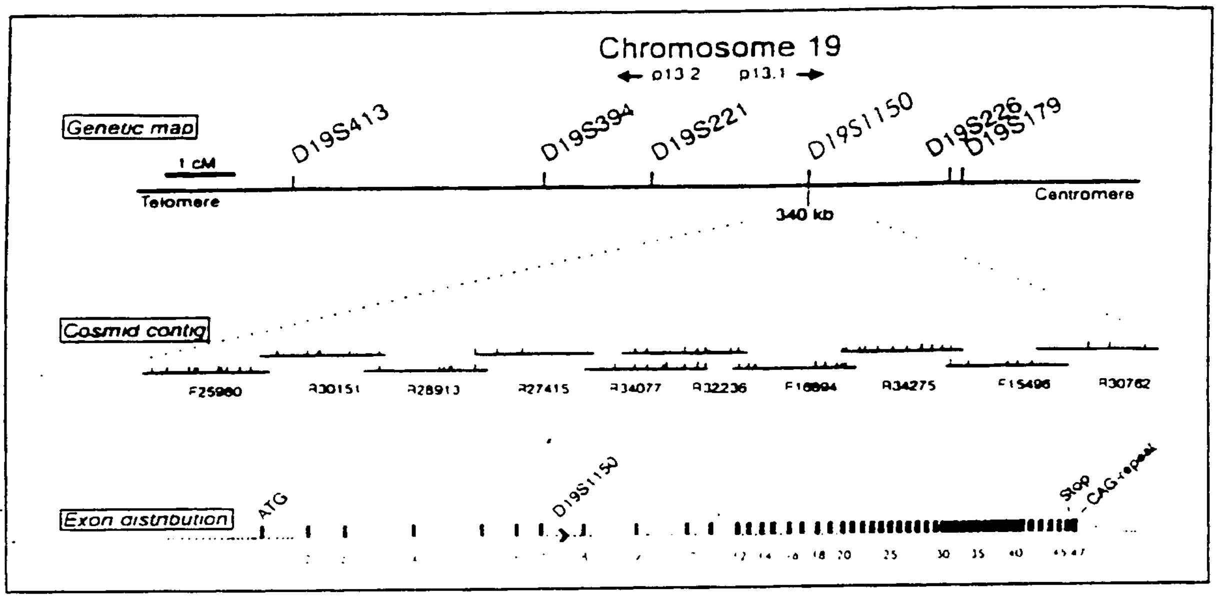
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p13.1
Genetic map
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D19S394
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D19S1150
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D19S179
Telomere
340 kb
Centromere
Cosmid contig
F25980
R30151
R28913
R27415
R34077
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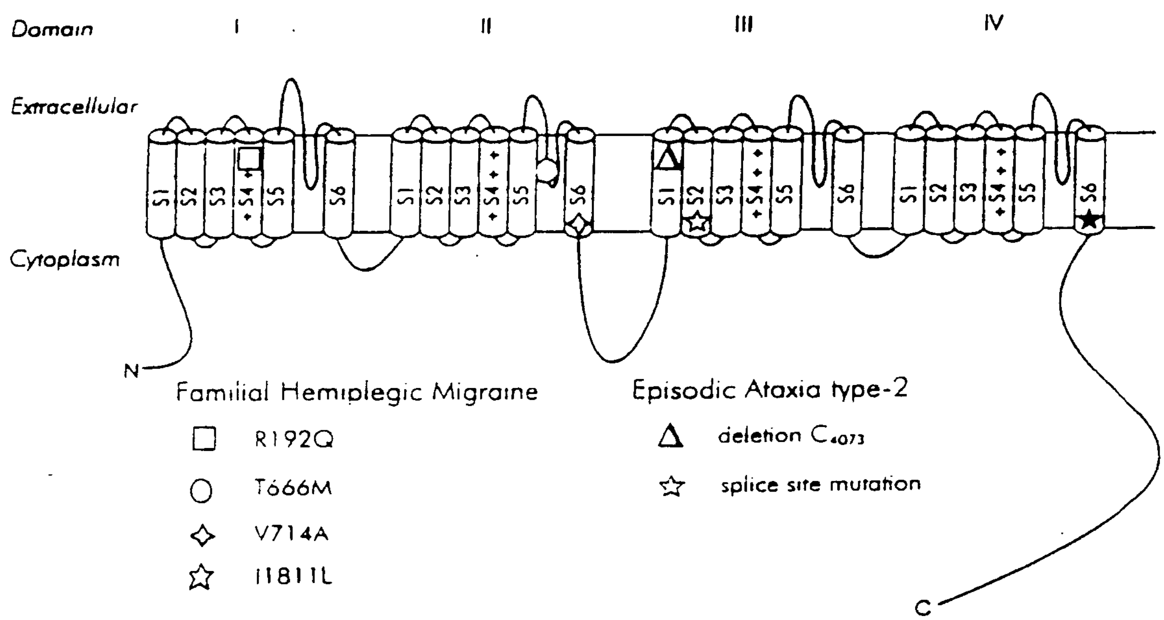
R34275
F15496
R30762
Exon distribution
ATG
D19S1150
Stop
CAG-repeat

Fig. 2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II
III
IV
Extracellular
Cytoplasm
S1
S2
S3
+S4+
S5
S6
N
C
Familial Hemiplegic Migraine
R192Q
T666M
V714A
I1811L
Episodic Ataxia type-2
deletion C4073
splice site mutation

Fig. 3

Figure 4

```
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A  G  V  V  V  G  S  G  G  G  R  G  A  G  G  S  R  Q  G  G      40
cagcccggggcgcaaaggatgtacaagcagtcaatggcgcagagagcgcggaccatggca    180
Q  P  G  A  Q  R  M  Y  K  Q  S  M  A  Q  R  A  R  T  M  A      60
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L  Y  N  P  I  P  V  R  Q  N  C  L  T  V  N  R  S  L  F  L      80
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E  Y  M  I  L  A  T  I  I  A  N  C  I  V  L  A  L  E  Q  H      120
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L  P  D  D  D  K  T  P  M  S  E  R  L  D  D  T  E  P  Y  F      140
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K  G  S  Y  L  R  N  G  W  N  V  M  D  F  V  V  V  L  T  G      180
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T  D  D  I  Q  G  E  S  P  A  P  C  G  T  E  E  P  A  R  T      280
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